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Protective effects of SCFAs on organ injury and gut microbiota modulation in heat-stressed rats

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Abstract

Purpose This research was conducted to investigate the potential of short-chain fatty acids (SCFAs) in protecting organs from heat stress-induced injuries and gut microbiota modulation.

Methods Sprague–Dawley rats were randomly assigned to various groups including a control group, a room temperature training group, a hyperthermia training group, SCFAs pretreatment group, and recipients received feces from the HT group. After strenuous training at high temperatures, the levels of plasma enzymes AST, ALT, BUN, and Cr were evaluated. The changes in gut microbiota and fecal metabolites were detected using 16S rRNA sequencing and GC–MS methods. Pathological examination of colon and liver tissues was conducted, and immunohistochemical techniques were employed to assess intestinal barrier function.

Results The findings indicate that SCFAs hold the potential for mitigating liver and colon damage caused by heat stress. With the intervention of SCFAs, there were observable changes in the structure and metabolites of the intestinal microbiota, as well as improvements in intestinal barrier function. Further support for the benefits of SCFAs was found through fecal microbiota transplantation, which demonstrated that modified gut microbiota can effectively reduce organ damage.

Conclusions This study provides evidence that SCFAs, as metabolites of the gut microbiota, have a valuable role to play in regulating gut health and mitigating the harmful effects of heat stress.

Keywords Short-chain fatty acids, Heat stress, Gut microbiota

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Background

Extreme heat events have become more frequent and longer-lasting in recent years (Meehl and Tebaldi 2004; Horton et al. 2016). Heat stress (HS) is a condition that influences human health. In severe cases, it may induce heat stroke, one of the most hazardous conditions that can cause high mortality without prompt treatment (Epstein and Yanovich 2019). HS can induce several diseases or physical damage in many people. As an example, classic heatstroke and exertional heatstroke are two types of heatstroke. Classic heat stroke often affects elderly individuals, chronically ill individuals, and children (Bouchama et al. 2007; Kravchenko et al. 2013; Byard 2013), and exertional heatstroke frequently impacts soldiers, athletes, and outdoor workers (Bouchama et al. 2022).

Reducing core body temperature is the cornerstone of treatment for HS-related diseases and physical damage (Casa et al. 2007). However, in many instances, there may be no time for treatment, and prevention is particularly important (Epstein and Yanovich 2019).

Gut injury plays one of the most vital roles in the pathology of HS-associated diseases. The reduced blood flow of the intestine influences cell viability, and the consequent oxidative and nitrosative stress leads to cell damage and the broken of the tight junctions between the epithelial cells. Consequently, bacteria and bacterial components such as LPS enter the systemic circulation and lead to endotoxemia (Lambert 2009; Epstein and Roberts 2011; Snipe et al. 2018). Several studies have demonstrated that the intestine may be an effective therapeutic target for HS-associated diseases (Bouchama et al. 2005; Andrade et al. 2015).

Gut microbiota and the host have a long co-evolutionary relationship, and gut microbiota plays crucial roles in the host's physiological functions. Unsurprisingly, it can also influence many pathological processes under different conditions (Hill and Round 2021; Aggarwal et al. 2023). Many studies have examined the relationship between gut microbiota or gut barrier and heat stress conditions and athletic performance. Exercise can influence the structure of gut microbiota, such as phyla Firmicutes, Bacteroidetes, and Proteobacteria, genera Bacteroides and Clostridium (Zhao et al. 2018a; Cronin et al. 2018), gut microbiota, in turn, can influence athletic performance (Hughes and Holscher 2021). In the case of HS, probiotics alleviated the adverse effects of the small intestine (Ashraf et al. 2013; Song et al. 2014), and Galacto-oligosaccharides (GOS) prevented the alternation of the tight junction of chickens under HS (Varasteh et al. 2015). The stability of gut microbiota is associated with excellent exercise performance (Furber et al. 2022). Veillonella has a relatively high abundance in elite athletes and can enhance exercise performance through lactate metabolism (Scheiman et al. 2019).

SCFAs are metabolites produced by gut microbiota. They can regulate host metabolism by signaling GPCRs (Priyadarshini et al. 2016; Ge et al. 2008). For example, SCFAs improve intestinal homeostasis by conferring anti-inflammatory properties and regulating the expression of REGIIIβ/γ and β-defensins (Wells et al. 2011; Zhao et al. 2018b). Furthermore, they can enhance gut barrier function by increasing tight junctions (Parada Venegas et al. 2019) and transepithelial electrical resistance (TEER) (Peng et al. 2009; Zheng et al. 2017).

However, no studies have been reported on SCFAs and HS, and we hypothesized that SCFAs might be capable of preventing HS-related diseases. The possible mechanism may involve sustaining the integrity of the gut barrier and modulating the gut microbiota and its metabolites.

Materials and methods

Animal management

Male Sprague–Dawley (SD) rats (age 6–8 weeks old, average weight 376.67 ± 19.28 g) purchased from Bengbu Inuojia Biotechnology Co., Ltd. were housed in the polyethylene cages separately with controlled temperature and humidity (room temperature 23 ± 1 °C, relative humidity 45% ± 2%) and 12 h light-12 h dark cycles, with free access to standard food and water for 1 week before the experiment. This animal study protocol was approved by the Ethics Committee of Huadong Medical Institute of Biotechniques (protocol code 1,105,002, May 2022).

Experimental design

A model of the intense exercise was established as follows: all SD rats were subjected to the daily treadmill (KW-PT, Nanjing Calvin Biotechnology Co. Ltd., Nanjing, China) in the humidity box (KW-SSC, Nanjing Calvin Biotechnology Co. Ltd., Nanjing China) running with progressive training for 5 days, starting with 10 min of running at 5 m min^{-1} and gradually increasing to 20 m min^{-1} to which the rats were adapted (temperature 20 ℃, humidity 45%).

Rats were randomly assigned to six groups in accordance with the random number table: control group (control, $n=5$), room temperature training group (RT group, $n=5$), hyperthermia training group (HT group, $n=8$), SCFAs pretreatment group (SCFAs group, $n=8$), recipients received feces from HT group, (HT. R group, $n=4$) and recipients received feces from SCFAs group, (SCFAs. R group, $n=4$). The control group received no treatment, RT group exercised on the treadmill at a normal temperature (23 ± 1 °C), HT group exercised on the treadmill at a high temperature (35 ± 1 °C). The SCFAs group was pretreated with SCFAs for 5 days and exercised on

the treadmill at a high temperature (35 ± 1 °C). The HT. R group and SCFAs. R group exercised on the treadmill at a high temperature (35 ± 1 °C). The running methods was running at a speed of 20 m min^{-1} on the treadmill. Adjust the thermostat installation guide for temperature and humidity. We initiated the experiment after 30 min, once the temperature and humidity had stabilized. The sign of exhaustion was that they were unable to continue, even with electrical stimulation.

The SCFAs group was pretreated with SCFAs (sodium acetate anhydrate 0.5535 g/100 ml, sodium butyric 0.285 g/100 ml, sodium propionate 0.384 g/100 ml) (Sangon Biotech Co., Ltd., Shanghai, China) in the drinking water for 5 days. The HT.R and SCFAs.R groups were administered intragastrically with a 5-day regimen of antibiotic-laden drinking water (vancomycin 100 mg/kg, Neomycin sulfate, metronidazole, and chloramphenicol 200 mg/kg). Then they were administrated orally with the feces from HT and SCFAs groups for 3 days. The experimental design was shown in a schematic diagram (Figure S1).

Pre- and post-exercise rectal temperature and running time were recorded. Exhausted rats were immediately sacrificed by severing the postcava under the anesthesia of 3% sodium pentobarbitone. All surgery was performed under sodium pentobarbitone, rats were monitored during the whole surgery process and all efforts were made to minimize suffering. Blood, colon, liver, and feces were harvested. The blood was centrifuged at 3000 rpm (4 °C, 10 min) to obtain serum. All samples were then frozen at − 80 °C.

Serum detection

Serum creatinine and urea nitrogen were measured with the Creatinine or Urea Nitrogen Test Kit (Nanjing Jiancheng Institute of Biological Engineering, Nanjing, China). Blood alanine aminotransferase (ALT) and aspartate transaminase (AST) concentrations were measured with the glutamic-pyruvic transaminase (GPT/ALT) and glutamic-oxalacetic transaminase (GOT/AST) Assay Kits (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China). Optical Density (OD) was detected by spectrophotometer/microplate reader.

Histological examination

Tissue samples from the intestines and liver were immediately fixed in 4% paraformaldehyde and stored for more than 24 h until pathological staining. The target tissue was removed from the fixative and placed in the dehydration box. Tissue specimens were dehydrated with a gradient of ethanol (75% ethanol for 4 h, 85% ethanol for 2 h, 90% ethanol for 2 h, 95% ethanol for 1 h, anhydrous ethanol I for 30 min, anhydrous ethanol II for 30 min, alcohol benzene for 5–10 min, xylene I for 5–10 min, xylene II for 5–10 min, paraffin I for 1 h, paraffin II for 1 h, paraffin III for 1 h) in the dehydrator and embedded in paraffin with an embedding machine before solidification. Sections with a thickness of 4-μm were obtained from a paraffin slicer after cooling at − 20 °C on a refrigerated table. Scoop up the sections with a slide from the tissue splitter and bake them in an oven at 60 °C. The prepared sections were stored at room temperature. Sections were dewaxed and hydrated with a gradient of ethanol (xylene I for 20 min, xylene II for 20 min, anhydrous ethanol I for 10 min, anhydrous ethanol II for 10 min, 95% ethanol for 5 min, 90% ethanol for 5 min, 80% ethanol for 5 min, 70% ethanol for 5 min, distilled water). Sections were stained with hematoxylin and eosin (H&E) to visualize nuclei and cytoplasm (sections were soaked in hematoxylin for 3–8 min, then washed with tap water, differentiated with 1% hydrochloric alcohol for several seconds, washed with tap water, returned blue with 0.6% ammonia water, and washed with running water, eosin for 1–3 min), and then dehydrated with a gradient of ethanol (95% ethanol I for 5 min, 95% ethanol II for 5 min, anhydrous ethanol I for 5 min, anhydrous ethanol II for 5 min, xylene I for 5 min, xylene II for 5 min). Tissue specimen sections were air-dried and then sealed with neutral gum. A fluorescence microscope was used to examine liver and colon samples. At a magnification of × 400, imaging of damaged tissue was obtained.

Immunohistochemical staining

Similar to H&E staining, liver and colon tissue specimens were fixed, dehydrated, embedded, and sliced. Paraffin sections with a thickness of 4 μm were baked in an oven at 65 °C for 1 h until dewaxed and hydrated, then washed three times with PBS for 5 min each. Sections were placed in EDTA antigen repair solution for high-pressure repair and washed three times with PBS for 5 min each after 10 min. Sections were soaked in 3% hydrogen peroxide solution for 10 min at room temperature to block endogenous peroxidase and washed three times with PBS for 5 min each. After drying, sections were sealed with 5% BSA to reduce nonspecific staining for 20 min. After removal of the BSA solution, 100 μL of the diluted primary antibody was added to each section to cover the tissue and stored overnight at 4 °C, followed by three washes with PBS for 5 min. After removing the PBS solution, 100 μL of ZO-1 (Proteintech, Chicago, USA) or Occludin antibody (Affinity Biosciences Pty Ltd., Melbourne, Australia) was added to each section and incubated for 30 min at 37 °C, followed by three washes with PBS for 5 min. After cleaning of the PBS solution, 100 μL of DAB solution was added to each section. Color rendering was controlled under a microscope. After completion of color

rendering, sections were rinsed with tap water, retaining the hematoxylin dye solution, removing the excess stain with 1% hydrochloric acid alcohol for 1 s, then rinsing with tap water, adding 0.2% ammonia, and rinsing with running water. Sections were dehydrated with gradients of alcohol (70–100%) for 10 min per gradient, then dried, soaked in xylene, and sealed with neutral resin. Images were observed with a light microscope.

Using ImageJ software, the cumulative optical density of the image was calculated from the intensity values of the yellow pixels in the yellow stained area. Mean density was used to indicate the relative expression of ZO-1 or Occludin, which was evaluated as the ratio of cumulative optical density to the measured area. Images must be captured under identical microscopic conditions, including a constant light source and exposure time. The mean optical density of multiple photographs per set was used to compute the mean and standard deviation.

16S rRNA gene sequencing

Bacterial DNA was obtained from the feces using the MagPure Soil DNA LQ Kit (Guangzhou Magen Biotechnology Co., Ltd., Guangzhou, China) according to the manufacturer's instructions. The purity and concentration of DNA were detected by agarose gel electrophoresis. The bacterial 16S rRNA gene was amplified for the hypervariable region V3–V4 with primer pairs: 343F (5′-TACGGRAGGCAGCAG-3′) and 798R (5′-AGGGTA TCTAATCCT-3′). Primers were connected with the Illumina sequencing adapter.

The quality of amplification was detected by electrophoresis. The PCR products were purified with Agencourt AMPure XP beads (Beckman Coulter Co., State of California, USA) and quantified with 1×dsDNA HS Assay Kit for Qubit (Yeasen Biotechnology Co., Ltd., Shanghai, China). The appropriate concentration was selected for sequencing. Sequencing was performed on an Illumina NovaSeq6000 (Illumina Inc., San Diego, CA; OE Biotech Company, Shanghai, China) with two paired-end read cycles of 250 bases.

Base calling analysis was used to transform the high-throughput sequencing raw data files into raw sequencing data. The data were stored in FASTQ format. Cut adapt software was used to cut the primers from the raw data. Qualified paired-end reads were filtered out of low-quality sequences, noise reduced, merged, and detected and cut off chimera reads with DADA2 using Qiime2 default parameters for quality control to obtain representative sequence and ASV abundance tables. The representative reads were screened using the Qiime2 package. All representative reads were annotated and blasted against Silva database Version 138 (16 s/18 s/ITS rDNA) using q2-feature-classifier with the default parameters. All representative sequences were compared with the Silva database version 138 for annotation. Beta diversity included principal component analysis (PCA), principal coordinates analysis (PCoA), and nonmetric multidimensional scaling (NMDS) for comparison between sample groups' differences. LEfSe analysis was used in the statistical analysis of microbial multivariate variables to estimate differences among microorganisms.

GC–MS analysis

Take 60 mg sample into 1.5 mL EP tubes, add two steel balls and 600 μL Methanol–water (volume ratio at 1:1, contain L-2-chlorphenylalanine,2 μg/mL). The grinder was at 60 Hz for 2 min after pre-cooling in the refrigerator at − 40 °C, then ultrasound extraction for 30 min in the ice-water bath. One hundred fifty microliters of chloroform was added into the sample with hybrid by vortex finder for 2 min. The ultrasound extraction is again, then place at – 40 °C for 30 min. Centrifuge for 10 min at 13,000 rpm, 4 °C. Transfer the supernatant (150 μl) into a glass vial, concentration centrifugal dryer dried the sample. Methoxamine hydrochloride in pyridine (80 μL, 15 mg/mL) was added into a glass vial, and then mixtures were vortexed for 2 min and incubated at 37 °C for 90 min. BSTFA derivatization reagent (50 μL), n-hexane (20 μL), and 10 kinds of internal standard (C8/C9/C10/C12/C14/C16/C18/C20/C22/C24, Chloroform configuration, 10 μL) were mixed and were added into samples. The mixture was vigorously vortexed for 2 min before being derivatized at 70 °C for 60 min. Keep at room temperature for 30 min before GC–MS analysis.

GC–MS Analysis was adopted with 7890B gas chromatography coupled with a 5977B MSD system (Agilent Technologies Inc., CA, USA). The system utilized a DB-5MS capillary column. Helium was used as the carrier gas with a flow rate of 1 mL/min. The temperature for the injector was maintained at 260 ℃. The injector volume was 1 μL without split injection. The initial oven temperature was held at 60 ℃ for 0.5 min, then raised to 125 °C at a rate of 8 °C/min, to 210 °C at a rate of 8 °C/min, to 270 °C at a rate of 15 °C/min, to 305 °C at a rate of 20 ℃/min, finally kept at 305 °C for 5 min. MS quadrupole and ion source temperatures were 150 °C and 230 °C. The collision energy was 70 eV. Mass spectrometric data were acquired in a full-scan mode (m/z 50–500). In the process of mass spectrum computerization, quality control samples consisting of sample mixtures in equal amounts were injected between samples at regular intervals to evaluate the stability of the mass spectrum platform of the system throughout the experiment.

Raw data from GC/MS in. D format were transferred to.abf format using Analysis Base File Converter software for quick data retrieval. After raw data

were imported into MS-DIAL software, peak detection, peak recognition, MS2Dec deconvolution, peak alignment, filtering, missing value interpolation, and a series of processing steps were performed on the data. Metabolites characterization was based on the LUG database (untarget database of GC–MS from Lumingbio) to derive the three-dimensional matrix, including sample information, the name of the peak of each substance, retention time, retention index, mass-to-charge ratio, and signal intensity. According to RSD (Relative standard deviation), less than 0.1 of internal standard which was used for quality control of the data, would be eliminated that included standard internal peaks, any known false positive peaks (noise, column loss, and derived chemical and physical reagent peaks) and the ion peaks of the missing value (0 value) greater than 50% in the group. Half of the minimum value was substituted for the remaining missing value (0 value). Each sample's peak signal intensity (peak area) was segmented and normalized based on the internal standard with RSD (ALL) less than 0.1 following the screening. After the data were normalized and then removed the redundancy and merged peak, compounds with qualitative results greater than or equal to 70 points were selected according to the qualitative results of compounds, and inaccurate data were deleted. Data matrix would be obtained. Unsupervised principal component analysis (PCA) was used to observe the population distribution among the samples and the stability of the whole analysis process. Supervised partial least squares analysis (PLS-DA) and orthogonal partial least squares analysis (OPLS-DA) were utilized to distinguish the differences in metabolism between groups. A sevenfold cross-validation method and 200 Response Permutation Testing (RPT) can be used to determine the quality of the model and avoid overfitting. Based on the OPLS-DA model, VIP (variable importance of projection) was used to select metabolites with greater than 1.0 values.

Statistical analysis

Data from detection were expressed as mean ± s.d.. Figures were performed using GraphPad Prism software version 5.0 for Windows (GraphPad Software INC, La Jolla, CA, USA). Plasma biochemical analysis was performed by Student's *t*-test. Kruskal–Wallis was used to analyze the different structures of gut microbiota between groups. The significance of metabolites difference between groups was verified using a two-tailed student's t-test. A two-tailed $P < 0.05$ was considered to declare a significant difference.

Results

SCFAs alleviate organ injury induced by exercise under high temperature in the rat

In our study, we employed rats as the animal model. We observed that the exercise duration time in the HT group was significantly shorter compared to the RT group. Interestingly, we discovered that SCFAs could enhance the exercise duration time under high-temperature conditions (as depicted in Fig. 1A). Additionally, we measured the rectal temperature, the control group was 36.8 ± 0.3 °C, the RT group was 40.1 ± 0.7 °C, the HT group was 41.5 ± 1.0 °C, the SCFAs group was 41.5 ± 0.7 °C. Both the RT and HT groups exhibited higher rectal temperatures compared to the control groups. However, there was no statistical difference observed between the SCFAs group and the HT group. It is noteworthy that SCFAs did not have a reducing effect on the rectal temperature, as demonstrated in Fig. 1B. Exercise at a high temperature may cause damage to multiple organs, as we found in colon and liver tissue. For the colon tissue, in the RT group, the local intestinal mucosa showed erosion and exfoliation. A small amount of lymphocyte infiltration and scattered nuclear debris can be found in the interstitium. In the HT group, intestinal mucosa was locally atrophied, accompanied by extensive mucosal necrosis and exfoliation, a large number of lymphocytes infiltrated in the interstitium, local muscular layer of the intestinal wall is thinning, submucosal edema, accompanied by vascular dilation and congestion. However, the SCFAs group did not show any abnormality (Fig. 1C).

For the hepatic tissue, in the RT group, the central vein showed dilation and congestion, the portal area showed a small amount of lymphatic infiltration, and hepatocytes showed mild edema. But in the HT group, the central vein showed congestion and edema, the structure of the hepatic vein was disorganized, the portal area showed lymphatic infiltration, and the steatosis and apoptosis of hepatocytes can be seen at high magnification, with more nuclear fragments. In the SCFAs group, the central vein was mildly dilated and congested, with a small amount of lymphocyte infiltration in the portal area, hepatocytes were slightly edema, and there was a small number of monocytes (Fig. 1D). We also detected ALT, AST, BUN, and creatinine serum concentrations. HT group showed the highest concentration of ALT and AST in the four groups. The SCFAs group showed lower concentration. BUN and creatinine showed similar variation tendency. The results showed the pretreatment of SCFAs could reduce the serum concentration of ALT, AST, BUN, and creatinine, but there was no statistical difference between the SCFAs group and the control group (Fig. 1E).

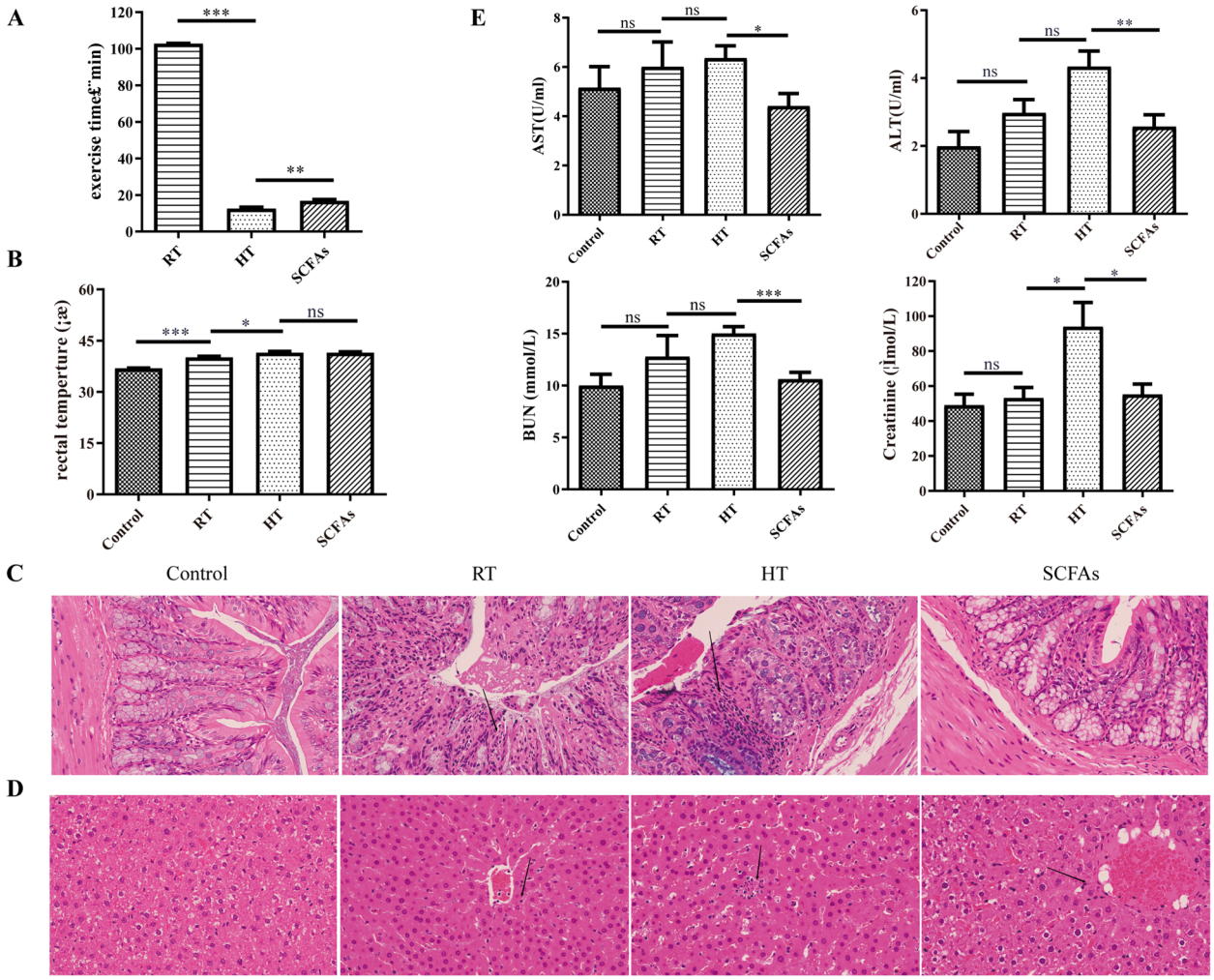

Fig. 1 Serum detection and histological examination of the colon and liver. SCFAs alleviate the organ injury induced by exercise under high temperature. **A** The exercise duration time in these groups. **B** The rectal temperature of these groups. **C** The HE staining of colon tissue of the control, RT, HT, and SCFAs groups. **D** The HE staining of hepatic tissue of the control, RT, HT, and SCFAs groups. **E** The serum concentration of ALT, AST, BUN, and creatinine

Change of gut microbiota during exercise and the pretreatment of SCFAs

We detected the gut microbiota of rats under different conditions. First, the RT group and HT group differed from the control group, and the HT group differed from the RT group (Figure S2). It demonstrated that exercise can alter the gut microbiota of rats, and exercise at a high temperature altered the gut microbiota even more than in the RT group. SCFAs were applied to observe their effect on exercise under high temperatures. We found the gut microbiota of the SCFAs group was different from the HT group. In the phylum level, the SCFAs showed a higher abundance of Bacteriodetes and a lower abundance of Firmicutes, and the F/B ratio was different between the two groups (Fig. 2A, B). In addition, PCoA and NMDS analysis techniques were used to determine the various global structures of SCFAs and HT groups (Fig. 2C, D). The heatmap in genus level showed the difference, too (Fig. 2E). Diverse bacteria at all classification levels are illustrated (Fig. 2F). For instance, in the SCFAs group, compared with the HT group, these bacteria showed higher abundance: phyla Bacteroides and Campilobacterota, genera Rodentibacter and Helicobacter. But in the HT group, for example, these bacteria showed higher quantity: phylum Firmicutes, class Coriobacteriia, Clostridia, genera Oscillibacter, Dubosiella, Oxalobacter.

Fecal metabolites shift during exercise and the pretreatment of SCFAs

The fecal metabolites were also detected using the GC–MS method. The RT group and HT group showed

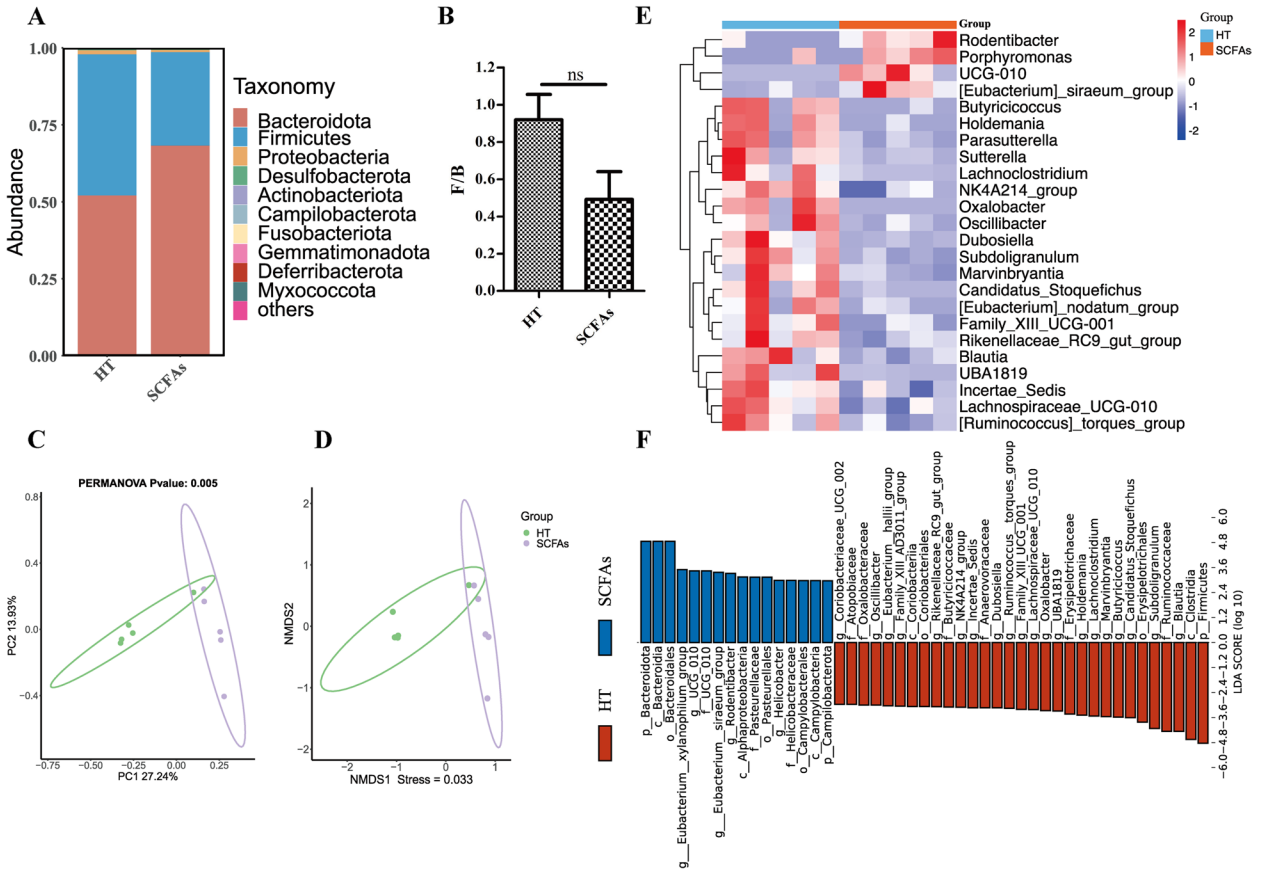

Fig. 2 16S rRNA gene sequencing. Change of gut microbiota between the SCFAs group and HT group. **A** the phylum level of the gut microbiota in the SCFAs group and HT group. **B** The F/B ratio between the SCFAs group and HT group. **C**, **D** The PCoA and NMDS analysis of the different overall structures of SCFAs and HT groups. **E** The heatmap in genus level. **F** The different bacteria in all classification levels

different metabolites compared to the control group, and the RT group differed from the HT group (Figure S3). In comparison to the HT group, the fecal metabolites were altered by SCFAs pretreatment. The PCA and OPLS-DA showed a significant difference in metabolites between the HT group and SCFAs group (Fig. 3A, B). The heatmap showed the different metabolites in these two groups (Fig. 3C). In the SCFAs group, the metabolites D-ribose 5-phosphate, mannose 6-phosphate, glucose-6-phosphate, D-maltose, alpha-lactose, trehalose, propionic acid showed higher abundance than the HT group. In the HT group, more metabolites showed a higher abundance than in the SCFAs group, such as urea, N-acetylaminoethanol, 3-hydroxymethylglutaric acid, piperidone, putrescine, uric acid, sorbitol, allantoin, hypoxanthine, oxoglutaric acid.

In our analysis, we utilized the KEGG database to identify different pathways. Compared with the HT group, the significantly up-regulated metabolite pathways were carbohydrate digestion and absorption, starch and sucrose metabolism, thyroid hormone synthesis, ABC transporters, basal cell carcinoma, lysosome, cholesterol metabolism, prolactin signaling pathway, insulin secretion, cortisol synthesis and secretion, fat digestion and absorption, cushing syndrome (Fig. 3D) Compared with the HT group, the significantly down-regulated metabolite pathways were purine metabolism, pyrimidine metabolism, central carbon metabolism in cancer, arginine biosynthesis, aminoacyl−tRNA biosynthesis, beta-alanine metabolism, glutathione metabolism, D-glutamine and D-glutamate metabolism, galactose metabolism, glycine, serine and threonine metabolism, phenylalanine metabolism, glyoxylate and dicarboxylate metabolism, glucagon signaling pathway, pantothenate and CoA biosynthesis, thiamine metabolism (Fig. 3E).

The gut microbiota of the SCFAs group alleviate the organ injury induced by exercise under high temperature through fecal microbiota transplantation

Fecal microbiota transplantation (FMT) was performed to demonstrate the roles of gut microbiota in the SCFAs pretreatment. The feces of the SCFAs group

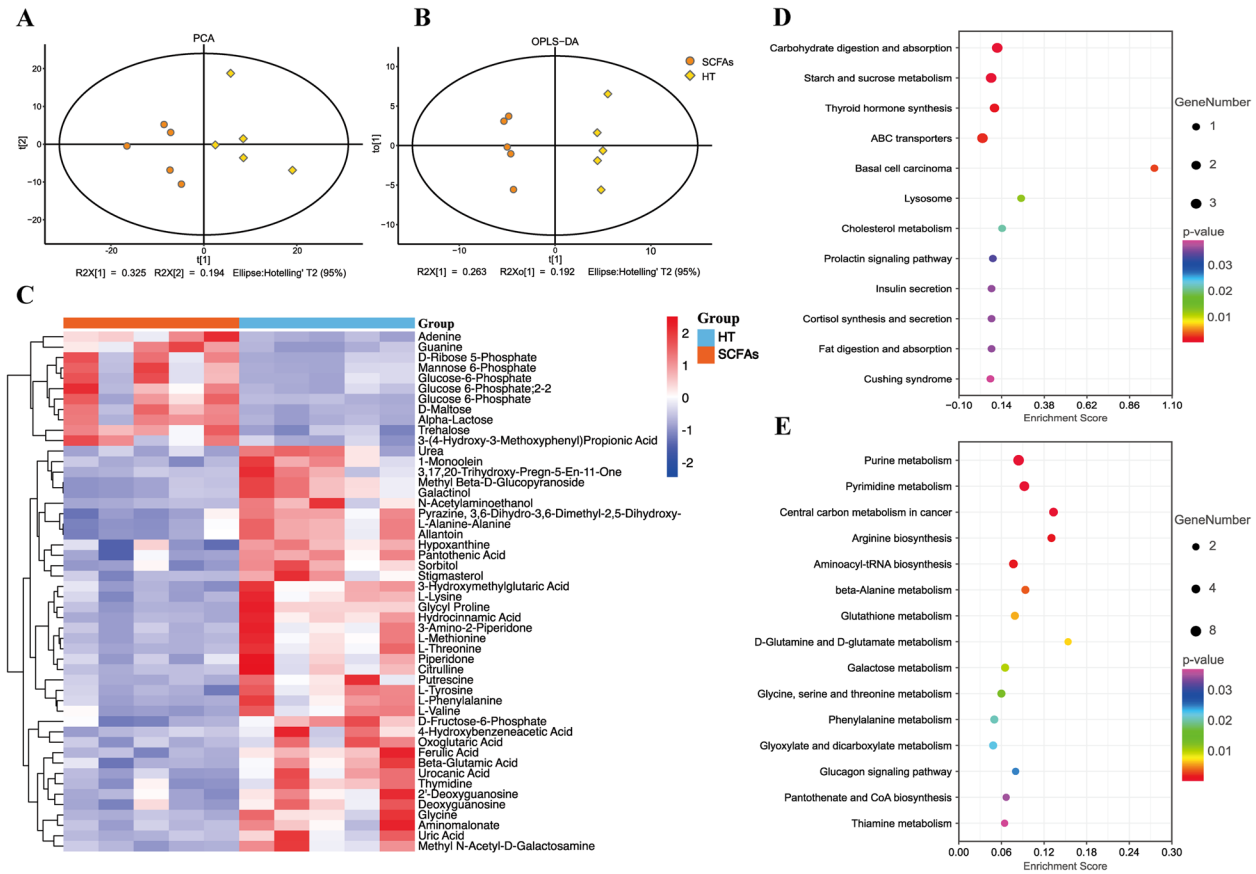

Fig. 3 GC–MS analysis. Change of fecal metabolites between the SCFAs group and HT group. **A**, **B** The PCA and OPLS-DA analysis between the HT group and SCFAs group. **C** The heatmap showed the different metabolites in the two groups. **D**, **E** The different pathways between the two groups according to the KEGG database

and HT group were administered to the recipients whose gut microbiota had been depleted with broad-spectrum antibiotics for 5 days. FMT lasted for 3 days, and then, the two groups of rats exercised under high temperature which is the same as the HT group. We found that the rats that received the feces of the SCFAs group had mild liver injury and colon injury compared with the rats that received the feces of the HT group (Fig. 4).

Pretreatment of SCFAs improved the gut barrier function which is destroyed in the HT group

We used the IHC method to detect the gut barrier-associated proteins such as ZO-1 and Occludin. We found the expression of Occludin and ZO-1 is higher in the SCFAs group than in the HT group (Fig. 5A, B). But the SCFAs.R group showed no statistical differences with the HT. R group (Figure S4). It showed that FMT may have little influence on the expression of Occludin and ZO-1.

Discussion

In this study, we discovered that SCFAs could improve the exercise performance of rats exposed to high temperatures and reduce liver and colon damage. In this process, the pretreatment of SCFAs changed the gut microbiota structure and the metabolites, in the SCFAs group, the gut microbiota showed a higher abundance of Bacteriodetes and a lower abundance of Firmicutes. The fecal metabolites and relevant metabolic pathways also varied between the HT group and the control group. SCFAs also improved the intestinal barrier function, it may be one of the mechanisms of the beneficial

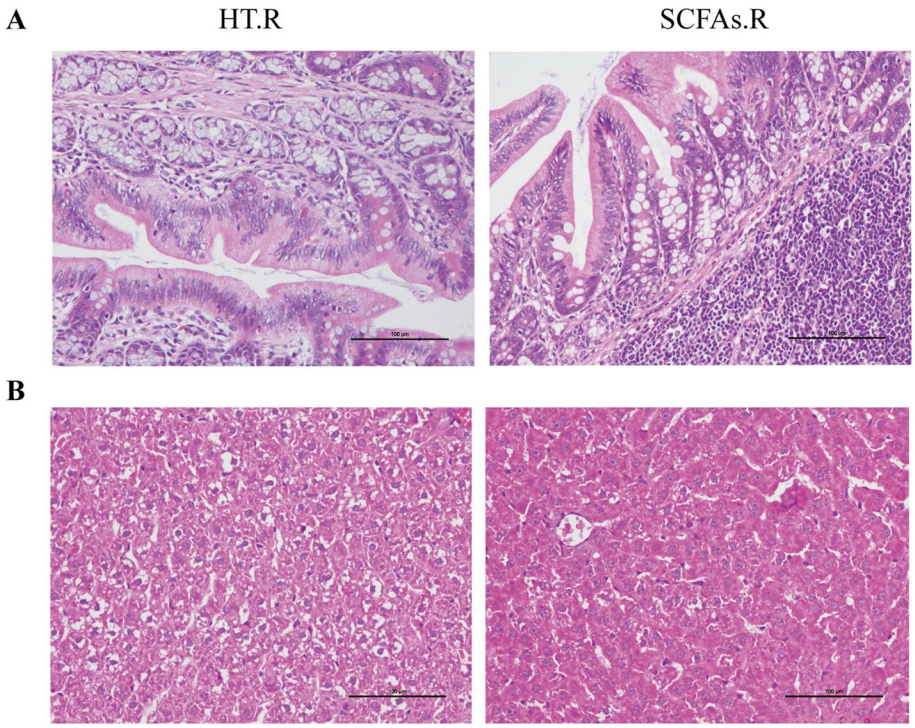

Fig. 4 Histological examination. The organ injury induced by exercise under high temperature was alleviated through FMT. **A**, **B** The HE staining of colon tissue and liver tissue of the HT. R and SCFAs. R groups

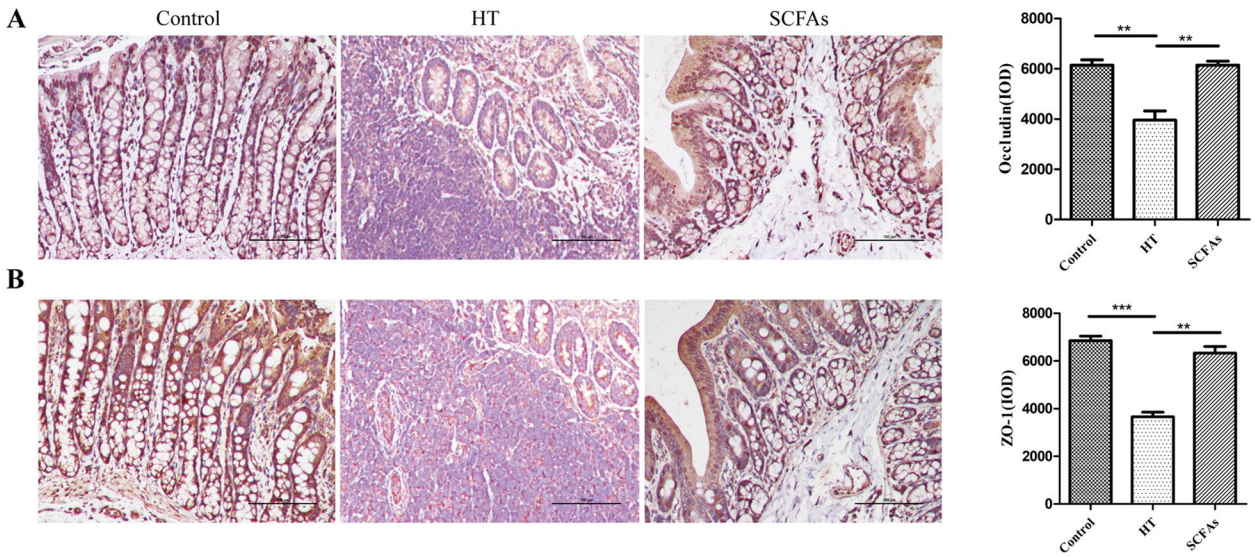

Fig. 5 Immunohistochemistry of Occludin and ZO-1. Pretreatment of SCFAs improved the gut barrier function. **A**, **B** ZO-1 and Occludin detection in the colon in HT group and SCFAs group

effects. To fully illustrate the influence of gut microbiota, we performed FMT. Although exercise time showed no difference, the liver injury and colon injury were alleviated through FMT. The findings supported the causal relationship between high-temperature exercise and SCFAs-influenced gut microbiota. This study laid the groundwork for future research on the specific bacteria and metabolites that can enhance exercise performance and reduce organ damage in high-temperature environments.

There are many studies about the gut microbiota, exercise, and heat stress. SCFAs are one of the mediators of energy metabolism in skeletal muscles (Clark and Mach 2017) and showed a high proportion after exercise (Allen et al. 2018). These studies demonstrated that SCFAs have an impact on muscles, which may be another reason why SCFAs could enhance exercise performance. Heat stress influences many organs and tissues including the kidney, liver, muscle tissue, and nervous system (Littmann and Shields 2016; Fan et al. 2015; Heneghan et al. 2014; Welc et al. 2013). These studies corroborate our findings, but they did not focus on the colon. In our study, we demonstrated that heat stress can also cause serious colon injury. During our research, we found SCFAs could reduce the concentration of ALT and AST, although the liver injury alleviation was less obvious than the colon alleviation, but the liver damage was alleviated contrasted with the HT group.

The gut microbiota can be modulated to exert a prevention effect on HS (Lian et al. 2020). Several studies have shown that probiotics and prebiotics are beneficial for HS due to their ability to maintain the integrity of the gut barrier and modulate immune function. For instance, a mixture of Bifidobacterium, Lactobacillus, and Streptococcus protected the gut barrier and reduced the serum LPS concentration under HS (Shing et al. 2014). It also demonstrated that the probiotics mixture can enhance the running time of male runners. Lactobacillus, a producer of SCFAs, may be linked to the beneficial effects observed, although this study did not prove this connection. We established the effects of SCFAs on SD rats, and perhaps in the future, similar effects may be demonstrated in humans. Bacilli subtilis improved the gut barrier and reduced the bacterial translocation of rats exposed to HS (Moore et al. 2014). B. licheniformis protected broiler chickens exposed to HS by reducing pro-inflammatory cytokines (Deng et al. 2012). Mannan-oligosaccharides (MOS) and cello-oligosaccharides (COS) alleviated the gut barrier injury induced by HS (Song et al. 2013; Sohail et al. 2012). Dietary Galactooligosaccharides (GOS) reduced the upregulation of IL-6 and IL-8 in the broiler chickens' jejunum under HS conditions (Varasteh et al. 2015). These studies demonstrated that probiotics and prebiotics can enhance gut barrier function and suppress the inflammatory response. However, the key difference in our study is that we proved that SCFAs possess both of these abilities.

Based on these studies, we observed that the intervention measures mainly consisted of traditional probiotics and prebiotics, lacking innovative approaches to modulate the gut microbiota for therapeutic or preventive purposes. In this study, we used SCFAs as the new intervention methods. The metabolites of gut microbiota received more and more attention from researchers due to the development of mass spectrum methods. An important function of the gut microbiota is the production of metabolites that contribute significantly to the health of the host. Our study showed that SCFAs prevented exercise-induced organ injuries under high-temperature conditions and improved athletic performance. Notably, SCFAs had similar effects to probiotics and prebiotics such as improving the gut barrier and modulating immune function (Huang et al. 2020; Wang et al. 2022; Holscher 2017). In light of these findings, it would be worthwhile to explore gut microbiota metabolites further in future studies, which would indicate that more research may be conducted in this area.

In this study, we discovered that the metabolite Trehalose was more prevalent in the SCFAs group. Trehalose was positively associated with the abundance of phylum Bacteroidota. It reduced oxidative stress and pro-inflammatory response (Luo et al. 2022), alleviated gut injury, and improved the integrity of the caecum epithelial cells induced by Salmonella Typhimurium infection (Mizunoe et al. 2018). But in the HT group, we found urea and uric acid had higher abundance. They are the key metabolites in the Purine metabolism pathway, and the Purine metabolism showed down-regulation in the SCFAs group. It has been shown the destruction of the gut barrier is associated with purine metabolism (Wu et al. 2020). These results showed the pretreatment of SCFAs could play their roles through the influence on the metabolites.

This study had certain limitations. First, the causal connection between pretreatment with SCFAs and exercise under HS conditions was not fully explained. The changed bacteria and metabolites need further research to confirm the specific bacteria strain or metabolite that affects HS. Second, SCFAs are a mixture of acetic acid, propionate acid, and butyric acid. In this study, we did not verify the roles of single short-chain acids. They may have a different effect on HS. SCFAs exert a preventive effect on HS. In this study, we aimed at the gut barrier and gut microbiota, but it is likely SCFAs may have other mechanisms to exert their beneficial effects. The precise molecular mechanism still needs to be determined. More research is needed to solve this problem.

Our study showed that exercise under HS conditions disrupts gut barrier function and alters the composition of the gut microbiome. It was found that pretreatment with SCFAs improved gut barrier function modulated the gut microbiota and associated metabolites, and alleviated liver and colon injuries resulting from exercise under high-stress conditions. In light of these findings, we propose a novel preventative approach to the treatment of heat-related disorders, or heat shock.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13213-023-01746-3.

Additional file1: Figure S1. The schematic diagram of the experiment. The schematic diagram showed the whole experimental design. **Figure S2.** The PCoA and NMDS analysis. The PCoA and NMDS analysis showed the four groups had different overall structures. **Figure S3.** The PCA and OPLS-DA analysis. The PCA and OPLS-DA analysis of the different fecal metabolites with the four groups. **Figure S4.** Immunohistochemistry of Occludin and ZO-1. Relative expression amount of Occludin and ZO-1 in HT. R group and SCFAs. R group had no significant difference.

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Authors' contributions
Conceptualization: ZY, FZ, and JZ. Experiment: CLT and XWS. Data curation: XWS, ZHW, XJZ, and RNZ. Funding acquisition: FZ and JZ. Project administration: CHW and FZ. Resources: JZ. Software: QC and XRZ. Supervision: YXS and YHM. Writing—original draft: ZY and CLT. Writing—review and editing: FZ and JZ.

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Availability of data and materials
The data presented in the study are deposited in the NCBI Sequence Read Archive (SRA) database, accession number PRJNA991337.

Declarations

Ethics approval and consent to particpate
This animal study protocol was approved by the Ethics Committee of Huadong Medical Institute of Biotechniques (protocol code 1105002, May 2022).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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References

Aggarwal N, Kitano S, Puah GRY, Kittelmann S, Hwang IY, Chang MW (2023) Microbiome and human health: current understanding, engineering, and enabling technologies. Chem Rev 123:31–72. https://doi.org/10.1021/acs.chemrev.2c00431
Allen JM et al (2018) Exercise alters gut microbiota composition and function in lean and obese humans. Med Sci Sports Exerc 50:747–757. https://doi.org/10.1249/mss.0000000000001495
Andrade ME et al (2015) The role of immunomodulators on intestinal barrier homeostasis in experimental models. Clin Nutr 34:1080–1087. https://doi.org/10.1016/j.clnu.2015.01.012
Ashraf S et al (2013) Effect of dietary supplementation of prebiotics and probiotics on intestinal microarchitecture in broilers reared under cyclic heat stress. J Anim Physiol Anim Nutr (berl) 97(Suppl 1):68–73. https://doi.org/10.1111/jpn.12041
Bouchama A et al (2022) Classic and exertional heatstroke. Nat Rev Dis Primers 8:8. https://doi.org/10.1038/s41572-021-00334-6
Bouchama A, Dehbi M, Mohamed G, Matthies F, Shoukri M, Menne B (2007) Prognostic factors in heat wave related deaths: a meta-analysis. Arch Intern Med 167:2170–2176. https://doi.org/10.1001/archinte.167.20.ira70009
Bouchama A et al (1985) (2005) Inflammatory, hemostatic, and clinical changes in a baboon experimental model for heatstroke. J Appl Physiol 98:697–705. https://doi.org/10.1152/japplphysiol.00461.2004
Byard RW (2013) Heat-related deaths. Forensic Sci Med Pathol 9:469–470. https://doi.org/10.1007/s12024-013-9413-y
Casa DJ, McDermott BP, Lee EC, Yeargin SW, Armstrong LE, Maresh CM (2007) Cold water immersion: the gold standard for exertional heatstroke treatment. Exerc Sport Sci Rev 35:141–149. https://doi.org/10.1097/jes.0b013e3180a02bec
Clark A, Mach N (2017) The Crosstalk between the Gut Microbiota and Mitochondria during Exercise. Front Physiol 8:319. https://doi.org/10.3389/fphys.2017.00319
Cronin O et al. (2018) A prospective metagenomic and metabolomic analysis of the impact of exercise and/or whey protein supplementation on the gut microbiome of sedentary adults. mSystems 3 https://doi.org/10.1128/mSystems.00044-18
Deng W, Dong XF, Tong JM, Zhang Q (2012) The probiotic Bacillus licheniformis ameliorates heat stress-induced impairment of egg production, gut morphology, and intestinal mucosal immunity in laying hens. Poult Sci 91:575–582. https://doi.org/10.3382/ps.2010-01293
Epstein Y, Roberts WO (2011) The pathopysiology of heat stroke: an integrative view of the final common pathway. Scand J Med Sci Sports 21:742–748. https://doi.org/10.1111/j.1600-0838.2011.01333.x
Epstein Y, Yanovich R (2019) Heatstroke. N Engl J Med 380:2449–2459. https://doi.org/10.1056/NEJMra1810762
Fan H et al (2015) Thrombocytopenia as a predictor of severe acute kidney injury in patients with heat stroke. Ren Fail 37:877–881. https://doi.org/10.3109/0886022x.2015.1022851
Furber MJW et al. (2022) Gut microbial stability is associated with greater endurance performance in athletes undertaking dietary periodization. mSystems 7:e0012922 https://doi.org/10.1128/msystems.00129-22
Ge H et al (2008) Activation of G protein-coupled receptor 43 in adipocytes leads to inhibition of lipolysis and suppression of plasma free fatty acids. Endocrinology 149:4519–4526. https://doi.org/10.1210/en.2008-0059
Heneghan HM et al (2014) Extreme heatstroke causing fulminant hepatic failure requiring liver transplantation: a case report. Transplant Proc 46:2430–2432. https://doi.org/10.1016/j.transproceed.2013.12.055
Hill JH, Round JL (2021) SnapShot: Microbiota effects on host physiology. Cell 184:2796-2796.e2791. https://doi.org/10.1016/j.cell.2021.04.026
Holscher HD (2017) Dietary fiber and prebiotics and the gastrointestinal microbiota. Gut Microbes 8:172–184. https://doi.org/10.1080/19490976.2017.1290756
Horton RM, Mankin JS, Lesk C, Coffel E, Raymond C (2016) A review of recent advances in research on extreme heat events. Current Climate Change Reports 2:242–259. https://doi.org/10.1007/s40641-016-0042-x
Huang WC, Pan CH, Wei CC, Huang HY (2020) Lactobacillus plantarum PS128 improves physiological adaptation and performance in triathletes through gut microbiota modulation. Nutrients 12 https://doi.org/10.3390/nu12082315
Hughes RL, Holscher HD (2021) Fueling gut microbes: a review of the interaction between diet, exercise, and the gut microbiota in athletes. Adv Nutr 12:2190–2215. https://doi.org/10.1093/advances/nmab077
Kravchenko J, Abernethy AP, Fawzy M, Lyerly HK (2013) Minimization of heatwave morbidity and mortality. Am J Prev Med 44:274–282. https://doi.org/10.1016/j.amepre.2012.11.015
Lambert GP (2009) Stress-induced gastrointestinal barrier dysfunction and its inflammatory effects. J Anim Sci 87:E101-108. https://doi.org/10.2527/jas.2008-1339
Lian P et al. (2020) Beyond heat stress: intestinal integrity disruption and mechanism-based intervention strategies. Nutrients 12 https://doi.org/10.3390/nu12030734
Littmann AE, Shields RK (2016) Whole body heat stress increases motor cortical excitability and skill acquisition in humans. Clin Neurophysiol 127:1521–1529. https://doi.org/10.1016/j.clinph.2015.11.001
Luo Z et al. (2022) Beta-glucan alters gut microbiota and plasma metabolites in pre-weaning dairy calves. Metabolites 12 https://doi.org/10.3390/metabo12080687
Meehl GA, Tebaldi C (2004) More intense, more frequent, and longer lasting heat waves in the 21st century. Science 305:994–997. https://doi.org/10.1126/science.1098704

Mizunoe Y et al (2018) Trehalose protects against oxidative stress by regulating the Keap1-Nrf2 and autophagy pathways. Redox Biol 15:115–124. https://doi.org/10.1016/j.redox.2017.09.007
Moore T, Globa L, Pustovyy O, Vodyanoy V, Sorokulova I (2014) Oral administration of Bacillus subtilis strain BSB3 can prevent heat stress-related adverse effects in rats. J Appl Microbiol 117:1463–1471. https://doi.org/10.1111/jam.12606
Parada Venegas D et al (2019) Short chain fatty acids (SCFAs)-mediated gut epithelial and immune regulation and its relevance for inflammatory bowel diseases. Front Immunol 10:277. https://doi.org/10.3389/fimmu.2019.00277
Peng L, Li ZR, Green RS, Holzman IR, Lin J (2009) Butyrate enhances the intestinal barrier by facilitating tight junction assembly via activation of AMP-activated protein kinase in Caco-2 cell monolayers. J Nutr 139:1619–1625. https://doi.org/10.3945/jn.109.104638
Priyadarshini M, Wicksteed B, Schiltz GE, Gilchrist A, Layden BT (2016) SCFA receptors in pancreatic β cells: novel diabetes targets? Trends Endocrinol Metab 27:653–664. https://doi.org/10.1016/j.tem.2016.03.011
Scheiman J et al (2019) Meta-omics analysis of elite athletes identifies a performance-enhancing microbe that functions via lactate metabolism. Nat Med 25:1104–1109. https://doi.org/10.1038/s41591-019-0485-4
Shing CM et al (2014) Effects of probiotics supplementation on gastrointestinal permeability, inflammation and exercise performance in the heat. Eur J Appl Physiol 114:93–103. https://doi.org/10.1007/s00421-013-2748-y
Snipe RMJ, Khoo A, Kitic CM, Gibson PR, Costa RJS (2018) The impact of exertional-heat stress on gastrointestinal integrity, gastrointestinal symptoms, systemic endotoxin and cytokine profile. Eur J Appl Physiol 118:389–400. https://doi.org/10.1007/s00421-017-3781-z
Sohail MU et al (2012) Effect of supplementation of prebiotic mannan-oligosaccharides and probiotic mixture on growth performance of broilers subjected to chronic heat stress. Poult Sci 91:2235–2240. https://doi.org/10.3382/ps.2012-02182
Song J et al (2013) Cello-oligosaccharide ameliorates heat stress-induced impairment of intestinal microflora, morphology and barrier integrity in broilers. Anim Feed Sci Technol 185:175–181. https://doi.org/10.1016/j.anifeedsci.2013.08.001
Song J et al (2014) Effect of a probiotic mixture on intestinal microflora, morphology, and barrier integrity of broilers subjected to heat stress. Poult Sci 93:581–588. https://doi.org/10.3382/ps.2013-03455
Varasteh S, Braber S, Akbari P, Garssen J, Fink-Gremmels J (2015) Differences in susceptibility to heat stress along the chicken intestine and the protective effects of galacto-oligosaccharides. PLoS ONE 10:e0138975. https://doi.org/10.1371/journal.pone.0138975
Wang J et al (2022) Dietary administration of Bacillus subtilis KC1 improves growth performance, immune response, heat stress tolerance, and disease resistance of broiler chickens. Poult Sci 101:101693. https://doi.org/10.1016/j.psj.2021.101693
Welc SS, Clanton TL, Dineen SM (1985) Leon LR (2013) Heat stroke activates a stress-induced cytokine response in skeletal muscle. J Appl Physiol 115:1126–1137. https://doi.org/10.1152/japplphysiol.00636.2013
Wells JM, Rossi O, Meijerink M, van Baarlen P (2011) Epithelial crosstalk at the microbiota-mucosal interface. Proc Natl Acad Sci U S A 108(Suppl 1):4607–4614. https://doi.org/10.1073/pnas.1000092107
Wu J et al (2020) Rhein modulates host purine metabolism in intestine through gut microbiota and ameliorates experimental colitis. Theranostics 10:10665–10679. https://doi.org/10.7150/thno.43528
Zhao X, Zhang Z, Hu B, Huang W, Yuan C, Zou L (2018a) Response of gut microbiota to metabolite changes induced by endurance exercise. Front Microbiol 9:765. https://doi.org/10.3389/fmicb.2018.00765
Zhao Y et al (2018b) GPR43 mediates microbiota metabolite SCFA regulation of antimicrobial peptide expression in intestinal epithelial cells via activation of mTOR and STAT3. Mucosal Immunol 11:752–762. https://doi.org/10.1038/mi.2017.118
Zheng L et al (2017) Microbial-derived butyrate promotes epithelial barrier function through IL-10 receptor-dependent repression of claudin-2. J Immunol 199:2976–2984. https://doi.org/10.4049/jimmunol.1700105

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