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Effect of exercise and butyrate supplementation on microbiota composition and lipid metabolism

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Abstract

The composition and activity of the gut microbiota depend on the host genome, nutrition, and lifestyle. Exercise and sodium butyrate (NaB) exert metabolic benefits in both mice and humans. However, the underlying mechanisms have not been fully elucidated. This study aimed to examine the effect of exercise training and dietary supplementation of butyrate on the composition of gut microbiota and whether the altered gut microbiota can stimulate differential production of short-chain fatty acids (SCFAs), which promote the expression of SESN2 and CRTC2 to improve metabolic health and protect against obesity. C57BL/6J mice were used to study the effect of exercise and high-fat diet (HFD) with or without NaB on gut microbiota. Bacterial communities were assayed in fecal samples using pyrosequencing of 16S rRNA gene amplicons. Western blot was performed using relevant antibodies to detect the protein expressions in liver and HepG2 cell extracts. Exercise and butyrate administration significantly reversed metabolic dysfunctions induced by HFD ($P < 0.05$). The number of Firmicutes and the proportion of Firmicutes to Bacteroidetes order were predominant in all HFD groups ($P = 0.001$). Exercise and butyrate supplementation significantly inhibited the relative abundance of lipopolysaccharide-producing phyla ($P = 0.001$). SESN2 and CRTC2 expression in the liver of mice were significantly increased after exercise ($P < 0.05$) and/or supplementation of butyrate ($P < 0.05$). Exercise enhances butyrate-producing fecal bacteria and increases butyrate production and consequently improves lipid metabolism through the butyrate-SESN2/CRTC2 pathway. Excess butyrate may reduce the proportion of probiotics and reverse the metabolic effects.

Key Words

- microbiota
- butyrate
- exercise
- HFD
- Sestrin2
- CRTC2

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Introduction

Symbiosis between the host and the intestinal microbiota plays a profound role in metabolism and the inflammatory response associated with the development of obesity (Lambertz *et al.* 2017). Recent evidence suggests that gut ecosystem development and its stability can be influenced by an existing dynamic balance between intrinsic and extrinsic factors that in turn can impact host health, such as diet and exercise (Monda *et al.* 2017). Food is the substrate for the growth of microbiota and directly affects its composition (da Silva *et al.* 2013). HFD induces obesity

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in isogenic C57BL/6J mice, partially by a progressive loss of diversity and dropout of major bacterial groups (Gerard 2016). However, feeding rodents a high level of dietary fiber protects against HFD-induced increases in body weight and fat mass (Lu *et al.* 2016). Because it is the main resource for production of endogenous SCFAs during bacterial fermentation in the colon, dietary fiber is deemed to be a key component in a healthy diet. Three predominant SCFAs, acetate, propionate, and butyrate, are typically found in a proportion of 1:1:3 in the intestinal tract (Thursby & Juge 2017). Butyrate is the primary energy source among the SCFAs, which circulates in the blood and can thus act on peripheral organs to modulate insulin sensitivity and the host's energy metabolism as a whole (Gao *et al.* 2009). Interestingly, dietary supplementation of butyrate has also been shown to protect against HFD-induced obesity, making butyrate a promising candidate in the prevention of metabolic disorders (Gao *et al.* 2009). In nature, the majority of butyrate is synthesized during anaerobic microbial fermentation of polysaccharides, indigestible to the host, which cannot produce any butyrate on its own (Stilling *et al.* 2016). Under physiological condition, butyrate is only derived from fermentation of dietary fiber in the gut and enters the circulation in variable m-molar concentration, butyrate mainly affects intestinal and adjacent tissues in a significant and mostly beneficial manner. Therefore, butyrate concentration usually varied in different tissues and organs (Canani *et al.* 2011). Excepting diet, exercise represents a cornerstone in the primary prevention of at least 35 chronic conditions (Campbell & Wisniewski 2017), but overall, how exercise influences microbiota is not fully understood. Several potential mechanisms by which exercise might alter the gut microbiota have been proposed. Exercise may impact the integrity of the gut mucus layer to keep microbes from adhering to the gut epithelium (Mailing *et al.* 2019). Most forms of aerobic exercise could increase GI transit, which has profound effects on intestinal pH, mucus secretion, biofilm formation, and availability of nutrients to microbes (Freeman *et al.* 2006). Lastly, exercise training may also alter the enterohepatic circulation of bile acids, which are potent regulators of gut microbiota community structure (Meissner *et al.* 2011).

Mice that performed physical exercise had levels of the Lactobacillales order up to 24 times higher than sedentary control (Choi *et al.* 2013), which could enhance the absorption of vitamins and have anti-obesity and anti-inflammatory effects. Growing evidence suggests that cross-talk between gut bacteria and host is achieved through specific metabolites like butyrate or molecular patterns of microbial membranes LPS that activate host cell receptors (Cani *et al.* 2014), but the exact mechanism remains unclear. Butyrate has also been shown to induce alterations of microbiota composition (Li *et al.* 2018), which suggests that butyrate may be a possible therapeutic factor in conjunction with exercise, diet, and other treatments. However, the precise nature of these associations is still unknown. We explored the comparative effects between host behaviors, including butyrate supplement and exercise, by investigating the biomarkers of inflammation and gut microbial ecology to identify an important target in the context of obesity.

Gut microbiome has received widespread attention due to its role in energy harvesting, and the microbiota mainly consists of bacteria from the well-studied two dominant phyla Firmicutes and Bacteroidetes (Cani & Delzenne 2007). In mice and humans, 16S ribosomal RNA gene sequencing of the colonic and fecal microbiome has linked obesity to an imbalance between the two dominant bacterial phyla, with a relatively low proportion of Bacteroidetes and a correspondingly higher proportion of Firmicutes (Turnbaugh *et al.* 2009). Our results suggested that the delicate balance between the key opportunistic pathogens, for example, *Enterobacter* spp. (Jiang *et al.* 2018) and favorable bacteria such as *Akkermansia* (Jiang *et al.* 2018) is especially critical for homeostasis.

Previous study demonstrated that obesity is associated with many pathological conditions and higher levels of inflammatory cytokines (Bastard *et al.* 2002). Inflammation and dysmetabolism appear to be consequences of obesity rather than its causes (Pina *et al.* 2015). Exercise is often prescribed for weight loss and its maintenance (O'Sullivan *et al.* 2015). Chronic exercise can reduce obesity by improving energy metabolism (Ringseis *et al.* 2015), probably due to the promotion of expression of Sestrin2. In mammals, the Sestrins family is composed of three genes – Sestrin1, Sestrin2, and Sestrin3. Among the three Sestrin subtypes, SESN2 has been suggested to inhibit pro-inflammatory gene expression induced by LPS (Yang *et al.* 2015). In addition, our previous study indeed demonstrated that long-term physical exercise significantly increased SESN2 (Wang *et al.* 2018) and had a protective role against obesity-induced insulin resistance (IR) by regulating cellular glucose metabolism homeostasis (Li *et al.* 2017). Beyond that, Wang *et al.* showed that CREB regulated transcription coactivator 2 (CRTC2) functioned as a mediator to maintain lipid homeostasis and directly affected lipid levels in mouse liver (Han *et al.* 2015).

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Previous research had demonstrated that high-intensity exercise can promote the expression of CRTC2 (Bruno *et al.* 2014). However, the relationship between regular aerobic exercise and CRTC2 is rather obscure. In this study, we found that 8-week aerobic exercise and butyrate can reverse metabolic endotoxemia and improve lipid metabolism through enhancing the expression of SESN2 and CRTC2 in mouse liver, which suggests that SESN2 and CRTC2 might be involved in mechanisms of lipid metabolism and regulated by chronic exercise and butyrate. We aimed to compare the effect of exercise and butyrate on host lipid metabolism and to what extent the two factors contribute to a beneficial metabolic state. We also investigated the nature of the underlying mechanism between intestinal microbiota and metabolic health.

Materials and methods

Animals and treatments

Four-week-old C57BL/6 male mice were housed in temperature-controlled (22 ± 2°C) quarters with a 12:12-h light–darkness cycle with free access to water and food. Mice were fed either (i) normal chow diet (NCD 70% of kilocalories from carbohydrate, 20% of kilocalories from protein, and 10% of kilocalories from fat, for a total energy content of 3.85 kcal/g) or (ii) HFD (35% of kilocalories from carbohydrate, 20% of kilocalories from protein, and 45% of kilocalories from fat, 4.73 kcal/g). Mice received NCD were divided into sedentary control group (NC) or exercise training group (NE) for 8 weeks. Under HFD conditions, mice were fed *ad libitum* and underwent one of four regimes: (1) HFD control group (HC); (2) HC with oral sodium butyrate group (HCB); (3) HFD with exercise training group (HE); or (4) HE with oral sodium butyrate group (HEB) for 8 weeks. Chronic exercise training was performed using a treadmill protocol as described previously (Wang *et al.* 2018). Administration of NaB (300 mg/kg/day) was modified in accordance with a previous study (Wang *et al.* 2017). The actual food intake was determined by measuring diet consumed in individual cages after normalizing diet spilled. Body composition was analyzed by dual-energy X-ray absorptiometry in isoflurane-anesthetized mice after 8 weeks. Blood samples were collected by orbital puncture. Mice were then killed by cervical dislocation. All animal protocols were approved by the Tianjin Medical University Animal Care and Use Committee under the guidelines of the Chinese Academy of Sciences.

Blood and tissue analysis

Blood and tissue samples were acquired 48 h after the end of last exercise to eliminate the transient effects of the exercise. Food were removed 13 h before sampling, the water supply was continued. All experimental animals' feces and blood samples were taken for butyrate analysis and the detection of other biochemical parameters. The abdominal visceral fat and liver were dissected and weighed, and tissues were then stored at −80°C for later analysis.

Chemicals and reagents

Butyric acid, sodium butyrate, and acetone were obtained from Sigma-Aldrich. Plasma triglyceride (TG) and total cholesterol (TC) concentrations were assayed by an enzymatic procedure (GPO-PAP) and the COD-CE-PAP method using triacylglycerol kit (Nanjing Jiancheng, China, A111-1) and total cholesterol assay kit (Nanjing Jiancheng, China, A110-1), respectively. Measurement of plasma free fatty acids (FFAs) and insulin concentrations were performed using FFA ELISA kit (EL701771-96T) and an insulin ELISA kit (EL-701891-96T). Examinations of plasma IL-1β, IL-6, and TNF-α were performed with the mouse IL-1β ELISA kit (EL701852), the mouse IL-6 ELISA kit (EL701689), and the mouse TNF-α ELISA kit (EL701678). Fecal and plasma SCFAs were measured using gas chromatography (GC) according to the method described previously (Wang *et al.* 2017).

Western blot analysis

Protein extracts from tissues were made in freshly prepared NP-40 lysis buffer. Equal amounts of protein were separated by SDS-PAGE and transferred to PVDF membranes (Millipore). The membranes were blocked and incubated overnight at 4°C with antibodies specific for Sestrin2, CRTC2, ACC, p-ACC-Ser79, FAS, CPT1, PPARα, and PPARγ (Cell Signaling Technology). All results are representative of three independent experiments.

Cell culture and treatments

HepG2, Hepa1-6 and THP1 cells were seeded into six-well cell culture plates and administrated with vehicle (as control), LPS (100 μg/mL), or LPS (100 μg/mL) + But (5 mM) for 24 h. Hepa1-6 cells were transfected with NC or Sesn2-siRNA (GenePharma, Shanghai, China) using Lipofectamine RNAiMAX (Invitrogen). For adenoviruses

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treatment, Hepa1-6 cells were infected with Ad-SESN2 using a multiplicity of infection (MOI) of 100 and harvested 48 h post infection.

Statistics

All experimental data were obtained from at least three independent experiments, and the data were expressed as mean ± standard deviation (s.d.). For data analysis, SPSS 22.0 software was applied by one-way ANOVA. $P<0.05$ was statistically significant.

Results

Effects of HFD and butyrate supplementation on body weight and energy metabolism after aerobic exercise in mice

Exposure to HFD for 8 weeks resulted in increased body weight gain ($P<0.05$) compared with NC group, but we found no significant difference between NC and NE group (Fig. 1A). HC+NaB had significantly restrained body weight increase from beginning to end, whereas that of HE occurred from week 2 onward and was significantly different (Fig. 1A). Interestingly, HE+NaB followed the same trend with HC+NaB and HE. Within HFD, HC had a higher energy intake than other groups, whereas chronic oral butyrate supplementation caused a sustained reduction in food and energy intake during the 8-week intervention period, HE showed no difference compared with HC group until week 8; however, within NCD, there was no significant difference between NC and NE groups (Fig. 1B and C). Butyrate supplementation decreased liver and the gonadal white adipose tissue (gWAT) pad weights by 29% (Fig. 1D) and 20% (Fig. 1E), respectively. Compared with those in the HC group, the liver and gWAT weights decreased by 16% (Fig. 1D) and 30% (Fig. 1E) respectively, in HE group. Consistent with our results, in a previous study, Li *et al.* showed that the anti-obesity action of butyrate was largely dependent on the reduction of food intake (Li *et al.* 2018). Considering the large variability observed in final body weight (and liver and fat mass) over the course of the study, we tested mice body composition and lipid metabolic parameters. After 8 weeks of intervention, HC group had greater BMI, body fat percentage, and markedly lower percentage of lean mass than all other groups. All three treatments protected against diet-induced obesity, with HE and HCB being more effective than HEB (Fig. 1H, I and J). Exercise significantly decreased plasma triglycerides (TG) and total cholesterol (TC) levels (Fig. 1K and L) and also markedly decreased FFA levels (Fig. 1M), whereas butyrate supplementation only decreased serum TG and TC.

Effects of HFD and butyrate supplementation on inflammation cytokine levels in serum after aerobic exercise in mice

HFD-induced metabolic endotoxemia and systemic low-grade inflammation play key roles in the pathogenesis of obesity. We detected the expression levels of endotoxemia and several important inflammatory cytokines in serum. ELISA results showed the LPS and pro-inflammatory

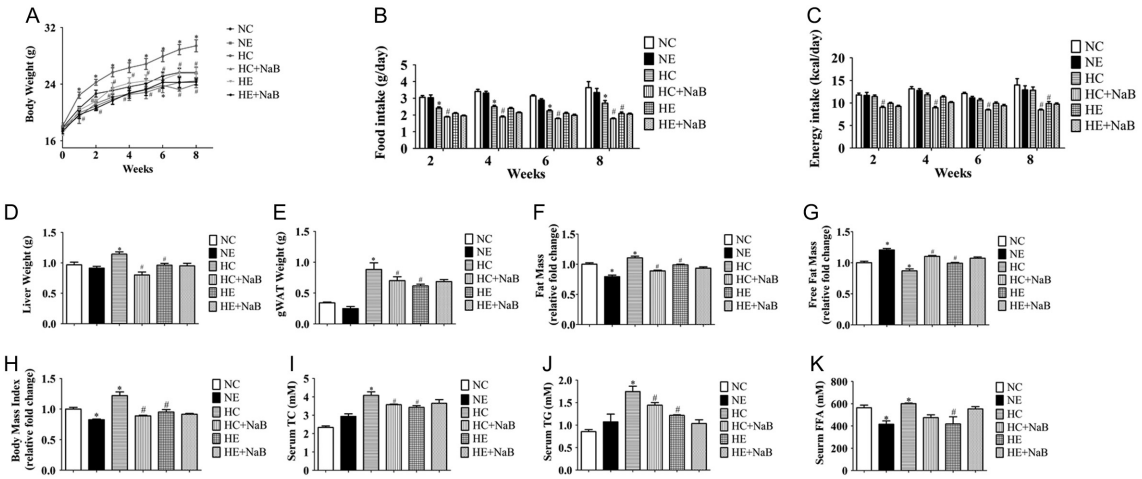

Figure 1
Effects of different interventions on mice metabolism. Body weight (A), food intake (B), energy intake (C), liver weight (D), gWAT weight (E), fat mass (F), free fat mass (G), BMI (H), TC (I), TG (J), and FFA (K) were determined. Data are shown as mean ± s.d. * vs NC, $P<0.05$; # vs HC, $P<0.05$.

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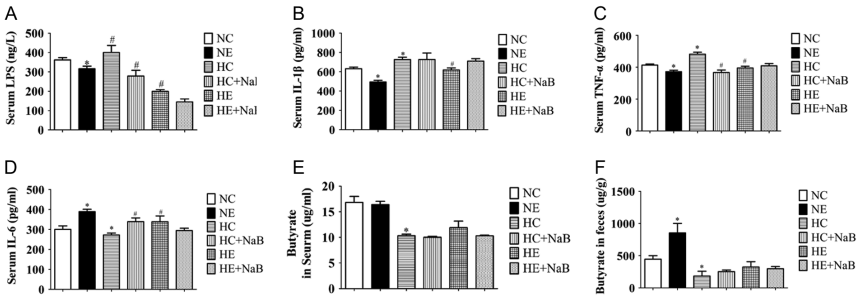

Figure 2
Effects of different interventions on metabolic endotoxemia and systemic chronic low-grade inflammation. Fasting plasma lipopolysaccharide (LPS) levels (A), cytokine concentrations in plasma (B, C and D). Butyrate in plasma and feces (E and F). Data are means ± S.D. * vs NC, $P < 0.05$; # vs HC, $P < 0.05$.

cytokines of TNF-α and IL-1β in serum significantly increased after 8 weeks HFD administration, which were partially reduced after exercise and/or butyrate supplementation. Among them, the improvement resulting from aerobic exercise was the most obvious (Fig. 2A, B, C and D). Interestingly, exercise and chronic butyrate consumption did not alter butyrate levels in peripheral blood, and only the HE group had significantly increased butyrate in fecal microbiome. However, combined therapy did not increase the level of butyrate in mice serum (Fig. 2E and F). The results indicated that exercise may increase the abundance of butyrate-producing fecal microbiome and that butyrate supplementation may affect microbiota composition.

Effects of HFD and butyrate supplementation on gut microbiota composition in fecal pellets from mice with/without aerobic exercise

The most important source for blood LPS is gut microbiota (Saad *et al.* 2016). As one of the metabolic products for microbiota, we intended to investigate if HE+NaB could yield better effects on composition of gut microbiota and host physiology. To further explore and compare the alteration of gut microbiota composition from different groups, the 16S rRNA genes of variable regions V3–V4 from the fecal samples of the six groups were sequenced by MiSeq platforms. As shown in Fig. 3A, ANOSIM analysis confirmed the effectiveness of intervention on intestinal microbiota ($P=0.001$). UniFrac-based principal coordinates analysis (PCoA) revealed a distinct clustering of microbiota composition for each group (Fig. 3B). A detailed overview of intestinal bacteria composition of each group is illustrated in Fig. 3C. By analyzing specific composition of microbiota, we observed that Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria are the four most important bacteria. Within chow diet, exercise markedly increased phylum levels of Bacteroidetes and decreased Firmicutes compared with NC group. Moreover, the pyrosequencing indicated that members of the Firmicutes and the proportion of Firmicutes to Bacteroidetes order were predominant in all HFD groups, which related to lipid synthesis. While the proportion of Firmicutes was significantly decreased in HE group, HC+NaB and HE+NaB simply followed this trend. In addition, the heat map of the top 20 bacterial genera that exhibited the most substantial change in abundance (Fig. 3D). Of the 20 genera, the abundance of eight genera increased after exercise training. Six of the eight genera were able to produce butyrate, including Enterobacter,

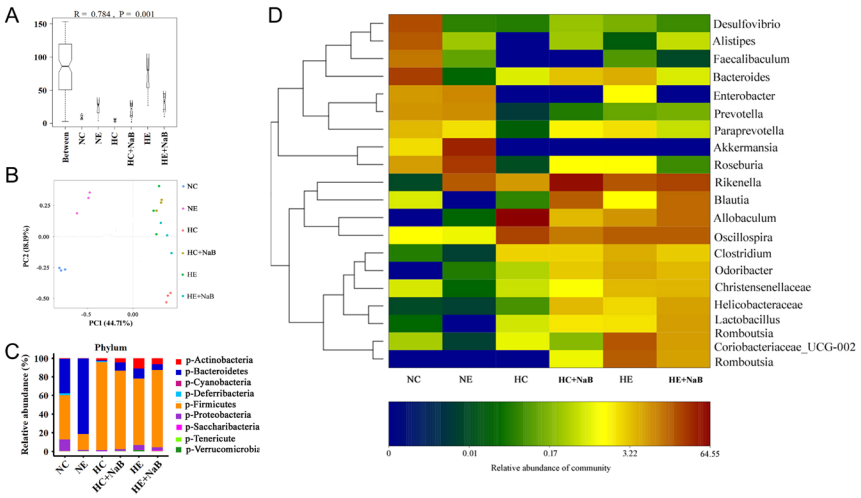

Figure 3
Effects of different interventions on gut microbiota composition. Anosim analysis confirmed the effectiveness of intervention on intestinal microbiota (A). Principal coordinate analysis (PCoA) and histogram for microbiota composition among groups (B and C). Heatmap of microbiota abundance (D). A full colour version of this figure is available at https://doi.org/10.1530/JOE-19-0122.

Bacteroides, Roseburia, Prevotella, Paraprevotella, and Akkermansia, with the biggest increase seen in Enterobacter and Akkermansia, specifically because of different diets. Furthermore, the HC group had a higher relative abundance of sequences belonging to Firmicutes, including Allobaculum and Oscillospira (Fig. 4). Linear discriminant analysis effect size indicated that the relative abundance of LPS-suppressing phyla Verrucomicrobia and the anti-inflammatory genus *Akkermansia* significantly increased compared with NC group. Furthermore, NCD groups had an obviously higher relative abundance of butyrate-producing bacteria and sequences belonging to Bacteroidetes, including Butyricicoccus, Bacteroides, Bacteroidia, Bacteroidales_S24-7_group, and Rikenella. Moreover, compared with HFD group, the relative abundance of Ruminococcaceae, which were reported to reduce HFD-induced obesity, were markedly increased in NCD groups. In addition, our data clearly indicated

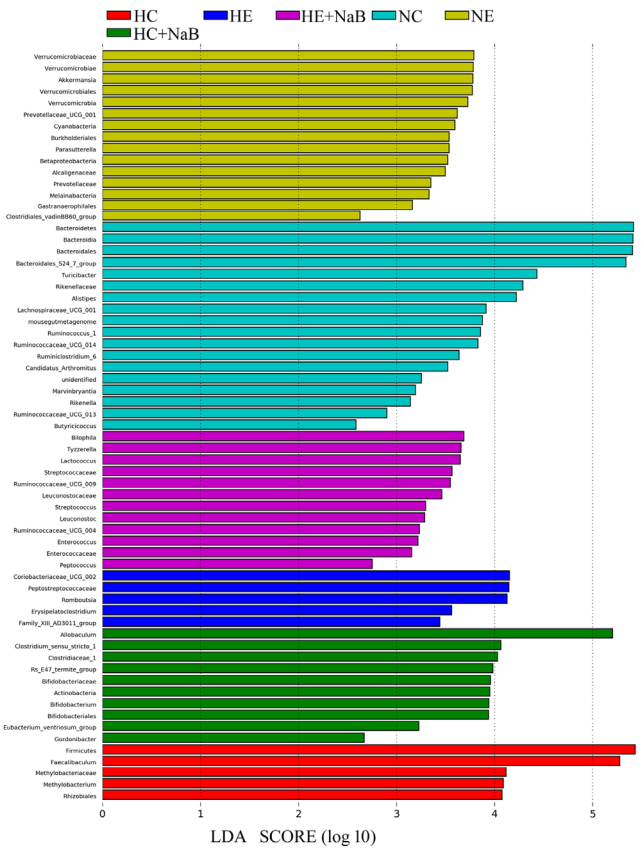

Figure 4
Linear discriminant analysis (LDA) score showing the most differentially significant abundant taxa enriched in microbiota from different groups. LDA score derived from LEfSe analysis, showing the biomarker taxa (LDA score of >2 and a significance of $P < 0.05$ determined by the Wilcoxon signed-rank test). A full colour version of this figure is available at https://doi.org/10.1530/JOE-19-0122.

that HFD alters the fecal microbiota composition, and in particular increases the abundance of the phylum Firmicutes, whereas dietary butyrate and exercise training markedly ameliorated HFD-induced body weight gain and lessened HFD-induced gut microbiota dysbiosis. HE significantly increased the level of Coribacteriaceae and Erysipclatoclostridium, which is associated with restoring liver steatosis. HC+NaB mainly increased Bifidobacteriaceae and Actinobacteria. Bifidobacteria had been reported to be reduced in obese mice, whereas Actinobacteria might be related to obesity and IR. Most of the bacteria added in HE+NaB consisted of conditional pathogens and a small number of probiotics.

Effects of HFD and butyrate supplementation on lipid metabolism after aerobic exercise in mouse liver

As illustrated in Fig. 5, HFD promoted the expression of lipid synthesis protein (ACC, p-ACC-Ser79, FAS) and decreased the expression of lipolysis protein (CPT1, PPARα, PPARγ) in mouse liver. As expected, all treatments tend to improve lipid metabolism to varying degrees among HFD. Given the pivotal roles of SESN2 and CRTC2 in energy metabolism, we detected the expression of the two molecules in mouse liver. Our result showed that HFD markedly upregulated the expression of SESN2 and downregulated the expression of CRTC2 compared to NC group. However, HE and HC+NaB both promoted the expression of two molecules in mouse liver, and the effect of HE group was the most obvious. The results suggested that SESN2 and CRTC2 may play important roles in lipid metabolism during exercise and butyrate treatment.

Mechanisms of butyrate on lipid metabolism

We found that exercise increases the number of butyrate-producing bacteria in the gut and increases butyrate levels in feces. Chronic exercise promoted the expression of SESN2 and CRTC2. Similarly, chronic butyrate supplementation also can promote expression of the two molecules. Therefore, we hypothesized that in part, exercise may have improved lipid metabolism through butyrate. LPS is well known as the major virulence factor of gram-negative bacteria (Hersoug *et al.* 2016). To verify the effectiveness of LPS-induced inflammatory response, we previously incubated THP1 cells in LPS and LPS+NaB for 24 h to detect the expression of inflammatory factors (Supplementary Fig. 1E, see section on supplementary data given at the end of this article). To comprehensively evaluate the capacity

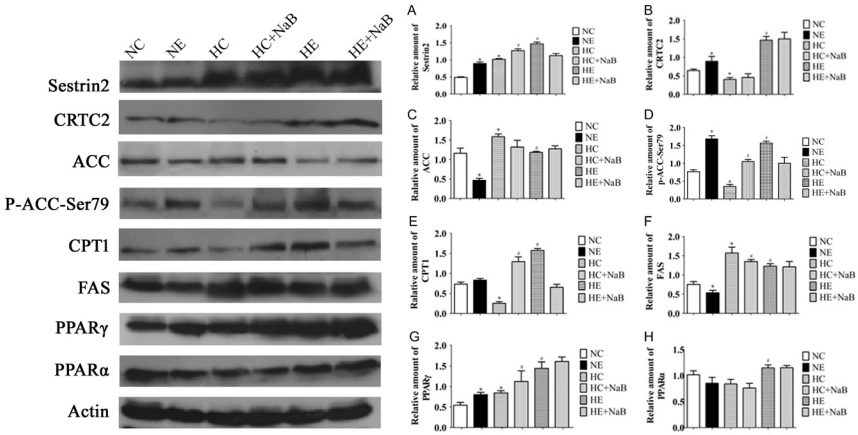

Figure 5
Effects of 8-week HFD and butyrate supplementation on lipid metabolism in mouse liver with/without aerobic exercise. Effects on Sestrin2 and CRTC2 expression (A and B). Effects on lipid synthesis proteins (C, D and E) and lipolysis proteins (F, G and H). Data are shown as mean ± S.D. * vs NC, $P < 0.05$; # vs HC, $P < 0.05$.

of butyrate to preserve LPS-induced dysmetabolism, HepG2 cells were treated with LPS followed by butyrate. Various concentrations of butyrate (1–10 mM) inhibit cell proliferation and induce apoptosis in multiple cancer cell lines (Zuo *et al.* 2013). In this experiment, HepG2 cell were treated with 5 mM butyrate according to the publication by Xu *et al.* (2018). As depicted in Fig. 6, the expression of inflammatory cytokines including IL-1β and TNF-α was strikingly promoted in LPS-treated group, while they were significantly inhibited in LPS-butyrate group. This finding indicates that butyrate may re-balance inflammatory cytokines. Similar to *in vivo* trial, LPS inhibited the protein expression of SESN2, CRTC2, and lipolysis protein (PPARα, PPARγ) while promoting the expression of lipid synthesis protein (p-ACC-Ser79, FAS). However, butyrate reversed the effects of LPS on HepG2 cells. To further investigate the regulatory effects of butyrate-SESN2/CRTC2 pathway on lipid metabolism, we generated Ad-SESN2, Sesn2-siRNA, and Crtc2-siRNA. Ad-SESN2 increased the protein level of Sestrin2 in Hepa1-6 (Supplementary Fig. 1A), whereas Sesn2-siRNA/Crtc2-siRNA decreased the protein level of Sestrin2/CRTC2 respectively (Supplementary Fig. 1C and D). Interestingly, LPS + NaB treatment increased the expression of Sestrin2 and CRTC2, whereas the combined use of LPS + NaB and Sesn2-siRNA decreased the expression (Fig. 7), indicating that Sestrin2 may regulated the expression of CRTC2 (Fig. 7A and B). In addition, LPS + NaB also increased the expression of lipolysis protein (CPT1, PPARα, and PPARγ) and decreased the expression of lipid synthesis protein (p-ACC-Ser79, FAS). However, LPS + NaB + Sesn2-siRNA reversed the effects of LPS + NaB on Hepa1-6 cells. To further demonstrate our hypothesis, Hepa1-6 cells were treated with LPS, LPS + Ad-SESN2 and LPS + Ad-SESN2 + Crtc2-siRNA for 24 h. We found that Ad-SESN2 increased the expression of Sestrin2 and CRTC2, while Crtc2-siRNA only inhibited the protein of CRTC2 and had no effects on Sestrin2 (G-H). In addition, LPS + Ad-SESN2 promoted the expression of lipolysis protein (CPT1, PPARα, PPARγ)

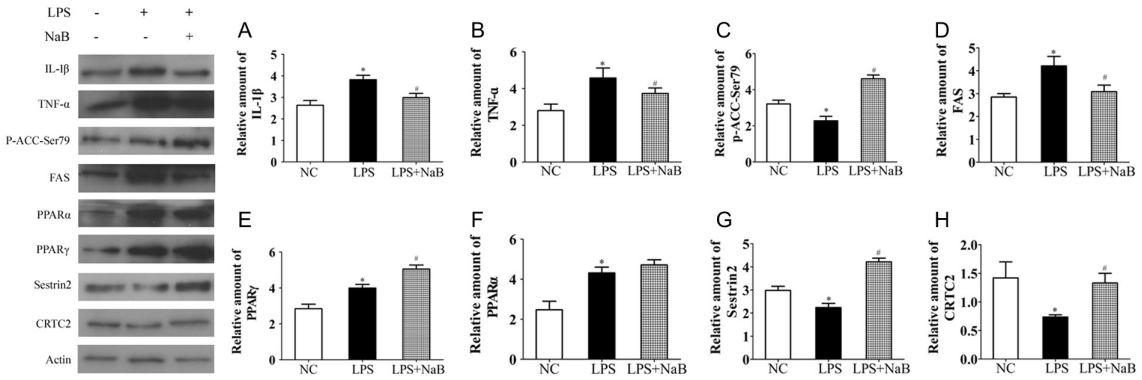

Figure 6
Effects of LPS and butyrate sodium on Sestrin2/CRTC2 signaling pathway in HepG2 cell line. HepG2 cells were incubated with vehicle, LPS (100 μg/ml) or LPS (100 μg/ml) + sodium butyrate (But, 5 mM/L) for 24 h. Inflammatory cytokines IL-1β (A), TNF-α (B), lipid synthesis protein (C and D), lipolysis protein (E and F), and protein SESN2, CRTC2 were determined by Western blot. Data are shown as mean ± S.D. * vs NC, $P < 0.05$; # vs HC, $P < 0.05$.

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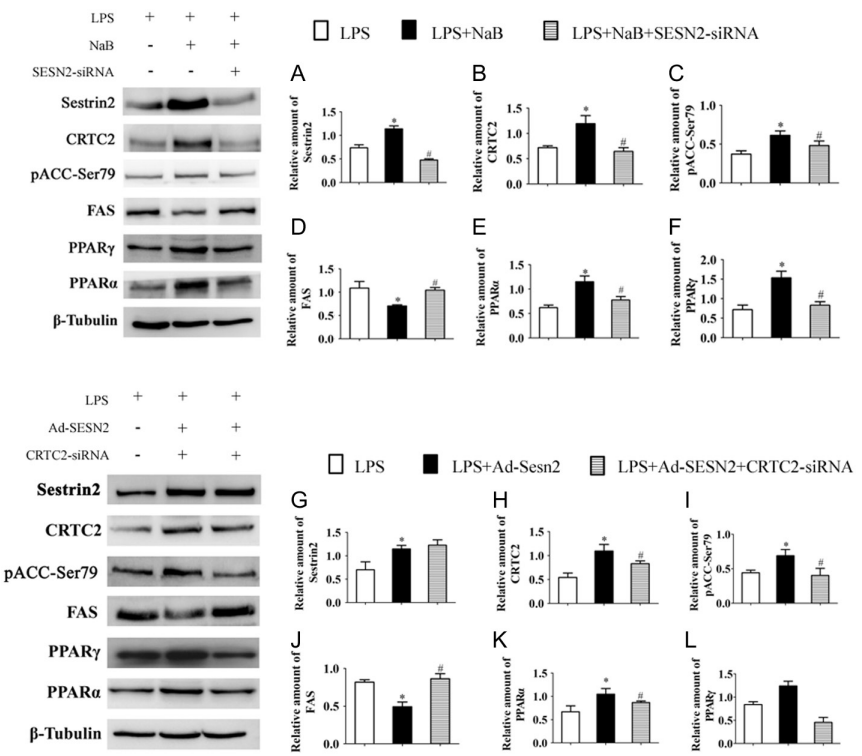

Figure 7
Butyrate improved lipid metabolism via regulating SESN2/CRTC2 pathway. Hepa1-6 cells were treated with LPS or LPS + NaB followed by transfecting with SESN2 adenovirus, Sesn2-siRNA, or Crtc2-siRNA for 24 hours. SESN2, CRTC2 protein (A, B and G, H), lipid synthesis protein (C, D and I, J) and lipolysis protein (E, F and K, L) were determined by Western blot. * vs LPS, $P < 0.05$; # vs LPS + NaB, # vs LPS + Ad-SESN2, $P < 0.05$.

and decreased the expression of lipid synthesis protein (p-ACC-Ser79, FAS), while LPS + Ad-SESN2 + Crtc2-siRNA reversed the effects of LPS + Ad-SESN2 on Hepa1-6 cells.

Discussion

Obesity and its associated disorders are a major public health concern. It is caused by many factors, including higher consumption of energy-rich diets and reduced physical activity. Over the past few years, the integral role of gut microbiota in the physiological regulation of host energy metabolism has attracted considerable attention. Several studies have revealed that obesity and metabolic disorders are associated with profound changes in gut microbiota (Bouter *et al.* 2017). However, mechanistic insights are currently lacking. Exercise can enhance butyrate-producing fecal bacteria. Meanwhile, butyrate supplementation is an important motivating factor that affects intestinal gut in a similar manner as exercise does, but the functional mechanism in regulating lipid metabolism was unclear. In particular, how microbiota-derived metabolites such as butyrate interact with host nutrient-sensing pathways to modulate energy metabolism is also poorly understood. Although it has been postulated that SCFAs regulate lipid metabolism, *in vivo* evidence was scant, and the downstream signaling pathway was not characterized. We addressed this issue in the present study and evaluated the preventive and therapeutic effects of different treatments against obesity.

This is the first study to compare the metabolic effects of exercise and butyrate, along with the liver SESN2 and CRTC2 response, in the context of different diets. In this study, we systematically examined the effects of three different treatments – oral butyrate, exercise training, and combined therapy – on lipid metabolism and microflora population. We found that all three treatments protected against diet-induced obesity, but HE and HC + NaB were more effective than HE + NaB. Butyrate at least partially regulates body weight by inhibiting food intake (Li *et al.* 2018), consistent with its prevented effects on body weight gain. In contrast, HE inhibited weight gain independent of food intake suppression and had obvious effects on gut microbiota. Note that mice-administered combined therapy exhibited increased levels of TC and TG. This was correlated with decreased levels of Firmicutes/Bacteroidetes, which confer beneficial metabolic effects and protect against obesity. In addition, HFD is associated with gut microbiota dysbiosis, which leads to increased blood levels of LPS toxins and pro-inflammatory cytokines. Both exercise and butyrate programs were able to prevent this dysbiosis but were more effective in HE group and had only minor effects in the combined therapy group. This suggests that butyrate is a double-edged sword

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regarding health. Excess butyrate could be transported via the portal vein to the liver, where it is involved in lipid biosynthesis and influences glycolipid metabolism (Liu *et al.* 2018). In fact, the role of butyrate in obesity remains controversial. Butyrate has been reported to alleviate diet-induced obesity and IR in mice (Hong *et al.* 2016). And dietary supplementation of butyrate prevented and reversed high-fat-diet-induced obesity by downregulating the expression and activity of PPARγ, promoting change from lipogenesis to lipolysis (den Besten *et al.* 2015). However, some studies showed an opposite effect *in vitro*. One study has shown that colonic cells exhibit a great capacity to rapidly oxidize butyrate into carbon dioxide through FA oxidation (Terova *et al.* 2016). In addition, butyrate is able to increase lipid synthesis from acetyl-CoA or ketone bodies via the β-hydroxy-β-methylglutaryl-CoA pathway, which potentially contributes to obesity (Birt *et al.* 2013). Therefore, although many evidences have suggested butyrate alleviates high-fat-diet-induced obesity and IR, its opposite effects on lipid metabolism were also reported. Our study showed that exercise or butyrate alone played an important role in inhibiting body weight gain, improving lipid metabolism and gut ecosystem in mice, while combination weakened their beneficial effects on metabolism. As mentioned in this study, exercise increases butyrate levels by increasing the abundance of butyrate-producing fecal microbiome. Excess butyrate may reduce the proportion of probiotics and reverse the metabolic effects. The usage and dosage of butyrate are critical in this process, and additional investigations are warranted to understand the apparently paradoxical effects of butyrate on obesity in future. Pathways that regulate energy homeostasis are a rational target for the development of novel therapies to treat obesity. Exercise-induced SESN2 mediated AMPK signaling and modulated the expression of metabolism target genes in our previous study (Liu *et al.* 2015, Li *et al.* 2017). Furthermore, Wang *et al*. showed that CRTC2 mediates SREBP1 activity and that hepatic triglycerides are increased by 50% in CRTC2-deficiency mice compared to WT mice fed with NC diet (Han *et al.* 2015). Interesting, Nelson showed that exercise promoted the expression of CRTC2 in skeletal muscle (Bruno *et al.* 2014). However, whether exercise improves lipid metabolism by CRTC2 is unclear. Currently, the relationship between SESN2 and CRTC2 is unexamined. It is known that exercise's anti-inflammatory properties help reduce low-grade inflammation. Inflammation is co-incident with obesity and diabetes, and it is plausible that this explains the observed reduction in the inflammatory response in HE group. Although we did not detect significant differences in food intake in exercise groups, we observed significant reductions in fat mass and body weight. We postulated that the increased metabolic rate may account for these effects. In all the training group levels of Firmicutes/Bacteroidetes decreased. The gastrointestinal tract is dominated by anaerobic bacteria belonging primarily to the three bacterial phyla: Firmicutes, Bacteroidetes, and Actinobacteria (Rook *et al.* 2017). More than 90% of the normal gut flora is represented by Firmicutes and Bacteroidetes phyla (Rook *et al.* 2017). Increased Firmicutes and decreased Bacteroidetes are associated with weight gain and IR (Baothman *et al.* 2016). These findings represent a bias in interpreting the degree of inflammation: typically, mice undergoing exercise training are more microbiota-homeostasis than sedentary mice groups. Interestingly, our results showed that exercise increased colonization of butyrate-producing bacteria and increased the levels of butyrate in fecal samples compared to HC group. Butyrate feeding decreased the Firmicutes and increased Bacteroidetes in the samples. We proposed that the beneficial effects of exercise may in part account for the increase in butyrate, which improves lipid metabolism in HFD-mice (Li *et al.* 2018). However, in HE+NaB group, chronic butyrate intervention weakened the beneficial effects of exercise on metabolism and microflora population. The results suggest that the usage and dosage of butyrate is a key point in the future development of metabolic drugs. In addition, we observed that exercise promotes the expression of SESN2 in mouse liver and may therefore protect lipid dysmetabolism from HFD. CRTC2 has been associated with lipid dysmetabolism and could be promoted by chronic exercise. However, little is known about the interactions between the gut flora and SESN2/CRTC2. Our study uncovered an exercise-butyrate-SESN2/CRTC2 pathway that confers protective metabolic effects. We found that exercise promotes the expression of SESN2 and CRTC2, which also showed increased expression upon butyrate treatment. Furthermore, a differential response between the two treatments was observed, in which exercise induced a further increase the expression of SESN2 and CRTC2 in liver. In addition, exercise modulated the gut flora composition (i.e. decreased Firmicutes and increased Bacteroidetes) and led to improved metabolic efficacy. The altered gut microbiota stimulates differential production of SCFAs (like butyrate) that in turn promotes the expression of SESN2 and CRTC2 to improve metabolic health and protect against

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obesity. Future studies will be necessary to determine the contributions of these mechanisms to energy homeostasis in the chronic setting and to assess if these findings hold any translational value.

Supplementary data

This is linked to the online version of the paper at https://doi.org/10.1530/JOE-19-0122.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

C X Y performed the experiments, analyzed and interpreted the data, and drafted the manuscript. L S J and L Q C performed part of the experiments and analyzed the data; J S, T Y W, Y M N, W Q Z analyzed data; L F critically revised the manuscript and was responsible for important intellectual content. All authors approved the final version for publication.

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