Bacillus amyloliquefaciens SC06 Protects Mice Against High-Fat Diet-Induced Obesity and Liver Injury via Regulating Host Metabolism and Gut Microbiota

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Obesity and the related liver diseases are prevalent around the world. Although probiotics have been shown to prevent obesity through multiple ways, only few researches investigated the lipid-lowering effects of probiotic Bacillus. Moreover, the limited results consistently suggested that Bacillus regulated genes related to lipogenesis and oxidation, but no further exploration was made. Our previous study revealed that Bacillus amyloliquefaciens SC06 has a potent antioxidant capacity in vitro.

The aim of this study is to investigate the effects of SC06 on obesity and the associated liver injury of high-fat diet (HFD)-fed-mice and its underlying mechanism. By feeding normal chow (NC), NC+SC06, HFD, and HFD+SC06 to mice, we found that SC06 improved body weight gain, hepatic steatosis, and glucose metabolism of HFD-mice. Furthermore, SC06 also increased the antioxidant capacity of mice through Nrf2/Keap1 signaling pathway. High-throughput sequencing of 16S rRNA gene showed that HFD changed the gut microbiota dramatically, while HFD+SC06 decreased the ratio of Firmicutes/Bacteroidetes and increased TM7 abundance. More differences were also found in lower taxa. Altogether, SC06 is a potential probiotic that decreases HFD-related lipid accumulation and liver injury via regulating the antioxidant capacity and host gut microbiota.

Keywords: Bacillus amyloliquefaciens, high-fat diet, liver injury, gut microbiota, oxidative stress

INTRODUCTION

With more than 1.4 billion overweight or obese adults (Hall et al., 2015), obesity has become a major threat to public health in both developed and developing countries (Haslam and James, 2005). In the United States, more than 36% of adults are obese with a body mass index (BMI) greater than 30 kg/m2 (Ogden et al., 2014). The mean BMI in Chinese adults also increased from 22.7 kg/m2 in
is worth noting that the lowered lipid accumulation induced by probiotics was found to be accompanied by the increase of antioxidant capacity (Do et al., 2015; Lei et al., 2015). Our previous study implied that Bacillus amyloliquefaciens SC06 (SC06) markedly elevated the antioxidant capacity of porcine intestinal epithelial cells (Wang et al., 2017). As oxidative stress is an obvious phenomenon in obesity, we hypothesis that SC06 may also prevent obesity and associated liver injury by regulating the antioxidant capacity and gut microbiota of hosts. In this study, we assessed the preventive effects of SC06 on HFD-induced obesity, liver injury and oxidative stress in mice and analyzed the intestinal microbiota structure.

MATERIALS AND METHODS

Bacteria

Bacillus amyloliquefaciens SC06 (SC06) cells were stored in China Center for Type Culture Collection (No. M 2012280). The culture and preparation of SC06 was referred to previous study (Wang et al., 2017). Briefly, SC06 powder (10^8 cfu/g) was prepared by Microbiology and Genetic Engineering Laboratory, Institute of Feed Sciences, Zhejiang University, China. SC06 was cultured on Luria-Bertani media, kept at 37°C for 24 h and shaken at 180 r/min. Pure bacterial cells were collected after centrifugation at 5000 g for 10 min at 4°C. Then, these cells were washed twice with sterile 0.85% sodium chloride solution. Ultimately, the culture purity and identification were constantly checked by the spreading plate method (Nikoskelainen et al., 2003).

Animals and Diets

The experimental procedure was illustrated in Supplementary Figure S1. Sixty male C57BL/6J mice (6 weeks old, n = 15 per group) were obtained from Slac Laboratory Animal Co., Ltd. (Shanghai, China) and fed on normal chow diet for 1 week to adapt to the environment. Thereafter, animals were divided into four groups and fed with normal chow (NC group, 3616 Kcal/Kg energy), NC supplemented with 0.1% (w/w) SC06 powder (NC+SC06 group), HFD (HFD group, 80% NC, 0.5% cholesterol, 6.3% lard, 13% dried egg yolk, and 0.2% cholate, 4270 Kcal/Kg energy) and HFD supplemented with 0.1% (w/w) SC06 powder (HFD+SC06 group) for 8 weeks. During the preparation of the SC06 powder, starch was used to dilute SC06 and the same amount of starch was also added to the NC and HFD groups to compensate for the difference in nutrient composition of the diets. Normal chow diet was purchased from Xietong Organism Co., Ltd. (Nanjing, China). The nutritional constitutes of HFD was based on previous study (Xin et al., 2014). NC+SC06, HFD, and HFD+SC06 diets were all prepared by Xietong Organism Co., Ltd. (Nanjing, China). Mice were housed in standard plastic cages (three mice per cage) and maintained under a 12-h light-dark cycle at constant temperature and humidity [(23 ± 1)-°C and (55 ± 5%), respectively]. Mice body weight and food intake were recorded. The mass of white fat, including the perirenal fat, subcutaneous fat and epididymal fat was weighed. The experiment was approved by and performed in accordance with the guidelines of the ethics committee of Zhejiang University.
**Insulin Sensitivity**

Oral glucose tolerance test (OGTT) and insulin tolerance test (ITT) were performed at the 7th week and 8th week, respectively. Before OGTT test, mice were fasted 8 h and were then given 2 g/Kg glucose orally. Blood glucose levels were determined with an Accu-chek glucose meter (Roche Diagnostics, Almere, Netherlands) at 0, 15, 30, 60, and 120 min. Before the ITT test, mice were fasted 4 h and insulin (0.75 U/kg) was injected intraperitoneally. Blood glucose levels were determined with an Accu-chek glucose meter (Roche Diagnostics, Almere, The Netherlands) at 0, 15, 30, 60, and 120 min.

**Western-Blotting Analysis**

Liver tissues were resuspended in lysis buffer (Biotime Biotechnology, China), ground and rocked for 30 min on ice. Crude lysates were then centrifuged at 12000 rpm for 10 min. Western-blotting analysis was performed according to previous study (Wang et al., 2017).

**Biochemical Evaluation of Serum**

The activities of glutamic-oxaloacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT), total-antioxidant capacity (T-AOC), superoxidase (SOD), and catalase (CAT) in serum were determined using commercial kits (Nanjing Jiancheng Bioengineering Institute, China) according to the instructions of the manufacturer. Interleukin (IL)-6, IL-1β, tumor necrosis factor (TNF)-α, and leptin concentrations were detected using a commercial ELISA kit (Raybiotech, GA, United States) according to the instructions of the manufacturer.

**Biochemical Evaluation of Liver**

T-AOC, CAT activities as well as malonaldehyde (MDA), GSH levels in livers were determined using commercial kits (Nanjing Jiancheng Bioengineering Institute, China) according to the instructions of the manufacturer.

**Hematoxylin and Eosin, and Oil Red O Staining**

Hematoxylin and eosin staining were performed according to previous study (Xin et al., 2014). For oil red O staining, 4-μm sections were cut from frozen optimal-cutting-temperature samples, affixed to microscope slides, and allowed to air-dry overnight at room temperature. The tissue sections were stained in fresh oil red O for 10 min and rinsed in water.

**Reactive Oxygen Species (ROS) Detection**

Frozen liver sections were incubated with 5 mol/L Dihydroethidium (Sigma-Aldrich, United States) for 30 min (37°C). After washes with PBS, the ROS level in the tissue was measured by confocal microscopy (Leica, Germany).

**TUNEL Staining**

The cell apoptosis in the liver was determined by TUNEL according to the manufacturer’s instructions In Situ Cell Death Detection Kit (KeyGen BioTECH, China).

**Transmission Electron Microscopy (TEM)**

Liver tissue was harvested after perfusion with PBS, then fixed and processed as described previously (Stolz et al., 1999). After dehydration, thin sections were stained with uranyl acetate and lead citrate for observation under a 1024 × 1024 pixel CCD camera system (AMT Corp., United States).

**Fecal DNA Extraction and Illumina Miseq Sequencing**

Genomic DNA was extracted using a TIANamp Stool DNA Kit according to manufacturer’s protocols (Tiangen Biotech, China). The V4 hypervariable region of 16S rRNA gene was amplified using the primer pair 515F (5′-GTGCCAGCMGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′), and MiSeq sequencing were performed by RiboBio Co., Ltd. (Guangzhou, China). All the DNA datasets have been submitted to the NCBI Sequence Read Archive database under the accession number SRP128678.

**Statistical Analysis**

The data are presented as the means ± SD with respect to the number of samples (n) in each group. Statistical significance between multiple treatment groups was determined using analysis of variance (ANOVA). Tukey’s method was used to perform multiple comparison of means. P-value less than 0.05 was considered as statistical significance.

**RESULTS**

**SC06 Treatment Attenuated Obesity and Insulin Resistance**

Compared with NC, NC+SC06 did not alter body weight significantly. HFD-fed mice gained more weight from week 5 than NC-fed mice (p < 0.05), but HFD+SC06 significantly relieved the HFD-induced body weight increases (p < 0.05, Figure 1A). Besides, the daily energy intake of mice in HFD group was increased compared with NC group (p < 0.05), while HFD+SC06 decreased energy intake significantly (p < 0.05, Figure 1B). Perirenal and subcutaneous partial fat weights were not changed in NC+SC06-fed mice compared to NC-fed mice, but were higher in mice receiving HFD (p < 0.05). Although weights of the perirenal, subcutaneous and epididymal fat were consistently decreased in HFD-fed mice received SC06, only subcutaneous fat weight reduced significantly (p < 0.05, Figure 1C). Furthermore, histological analysis of perirenal, subcutaneous and epididymal adipose tissues showed that the size of adipocytes in the HFD group was greater than that in NC, but the size of adipocytes in the HFD+SC06 group was less than that in HFD group (Figures 1D–F). In addition, HFD significantly increased glucose levels during the OGTT (p < 0.05, Figure 1G), and HFD+SC06 elevated the clearance of glucose slightly. Moreover, HFD worsened the insulin resistance
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FIGURE 1 | SC06 attenuated HFD-induced obesity, insulin resistance and hepatic steatosis. (A) Body weight, (B) daily energy intake, (C) partial white fat mass, (D) subcutaneous adipocyte morphology, (E) perirenal adipocyte morphology, (F) epididymal adipocyte morphology, (G) OGTT and ITT, AUC means area under the curve. (H) HE liver sections, (I) oil-red O liver sections. Results are expressed as means ± SD (n = 15 for body weight, energy intake, and fat mass; n = 4 for tissue morphology). Differences between groups were determined by one-way ANOVA followed by Tukey’s t-test. Significant differences between HFD versus NC are indicated as *p < 0.05. Significant differences between HFD versus HFD+SC06 are indicated as #p < 0.05.
slightly, while HFD+SC06 marginally improved the insulin sensitivity ($p = 0.058$).

**SC06 Treatment Alleviated Liver Steatosis and Injury of HFD-Induced Mice**

The HE and oil-red O liver sections from HFD mice exhibited visible intracellular vacuolization and marked lipid accumulation. Compared with the HFD group, the degree of hepatic steatosis was appreciably decreased by SC06 treatment (Figures 1H,I). The results of GOT and GPT activities showed that HFD or/and SC06 had no significant effect on GOT activity. However, GPT activity was significantly enhanced in HFD group ($p < 0.01$), but decreased slightly in HFD+SC06 (Figure 2A). Additionally, TUNEL results indicated that HFD enhanced liver cell apoptosis, which was reduced by SC06 treatment (Figure 2B). The mitochondrial TEM revealed that HFD seriously induced mitochondrial abnormalities, including swelling and disappearance of cristae, which were effectively alleviated by administration SC06 (Figure 2C).

**SC06 Treatment Decreased the Secretion of Inflammatory Factors and Adipokine of HFD-Induced Mice**

No significant changes were found for serum levels of IL-6, IL-1β, TNF-α and leptin between NC and NC+SC06 groups (Figures 3A–D). As expected, leptin, IL-6 as well as TNF-α levels were significantly elevated in HFD group ($p < 0.05$), but lowered in HFD+SC06 (Figures 3A–C) ($p < 0.05$). However, IL-1β level was not altered significantly in HFD and/or HFD+SC06 groups (Figure 3D).

**SC06 Relieved Liver Oxidative Stress of HFD-Induced Mice**

Table 1 shows that mice fed with NC+SC06 exhibited higher hepatic CAT activity ($p < 0.05$). Whereas, NC+SC06 had no significant effects on MDA, T-AOC, GSH levels and SOD activity in liver. HFD significantly increased MDA content ($p < 0.05$), which was lowered significantly by HFD+SC06 ($p < 0.05$).

Nrf2-Keap1 is a well-characterized pathway that responds to oxidative stress and regulates genes of phase II detoxifying enzymes through binding with antioxidant-responsive element (Turnbaugh et al., 2008; Jones et al., 2015). Figure 4A shows that Nrf2 expression was marginally increased in mice feeding NC+SC06 ($p = 0.054$). Meanwhile, the phosphorylation of Nrf2 was significantly up-regulated in HFD group compared to NC ($p < 0.05$). Compared to HFD, mice receiving HFD+SC06 showed down-regulated p-Nrf2 expression ($p < 0.05$). No significant differences were observed in Keap1 expressions among all groups (Figure 4A).

Oxidative stress is derived either from an increase in ROS production or decreased levels of ROS scavenging. According to Figure 4B, HFD elevated ROS levels in liver ($p < 0.05$), but HFD+SC06 inhibited the elevated ROS...
FIGURE 3 | SC06 modulated secretion of inflammatory factors and adipokine. (A) Leptin, (B) IL-6, (C) TNF-α, (D) IL-1β. Results are expressed as means ± SD. Differences between groups were determined by one-way ANOVA followed by Tukey’s t-test (n = 6). Significant differences between HFD versus NC are indicated as *p < 0.05, **p < 0.01. Significant differences between HFD versus HFD+SC06 are indicated as #p < 0.05, ##p < 0.01.

TABLE 1 | HFD and SC06 effects on liver antioxidant parameters.

| Parameters     | NC      | NC+SC06 | HFD   | HFD+SC06 |
|----------------|---------|---------|-------|----------|
| MDA (nmol/mg prot) | 2.66 ± 0.22 | 2.57 ± 0.13 | 3.59 ± 0.17* | 2.36 ± 0.05# |
| T-AOC (U/mg prot) | 0.49 ± 0.01 | 0.56 ± 0.12 | 0.46 ± 0.13 | 0.57 ± 0.04 |
| GSH (mg/g prot) | 1.55 ± 0.32 | 1.52 ± 0.32 | 1.45 ± 0.31 | 1.23 ± 0.23 |
| CAT (U/mg prot) | 13.84 ± 1.73 | 17.06 ± 2.14* | 15.27 ± 1.27 | 16.66 ± 1.71 |
| SOD (U/mg prot) | 141.67 ± 27.59 | 135 ± 12.42 | 157.03 ± 26.61 | 166.52 ± 21.89 |

Results are expressed as means ± SD. Differences between groups were determined by one-way ANOVA followed by Tukey’s t-test (n = 6). Significant differences between HFD versus NC are indicated as *p < 0.05. Significant differences between HFD versus HFD+SC06 are indicated as #p < 0.05.

concentration induced by HFD, although this was not statistically significant (p = 0.06).

Overall Structural Modulation of Gut Microbiota After SC06 Treatment

α-diversity (richness and evenness) of the communities was measured by Simpson’s, Goods_coverage’s, Chao1’s and Observed_species’ indexes, respectively (Figure 5). After 2- and 4-week HFD feeding, α-diversity was much higher than that of NC (p < 0.05). However, at the 8th week, there was no difference between the two groups (Figures 5A,B,D). In addition, as shown by the Simpson’s index, we observed that HFD+SC06 increased the α-diversity obviously at the 2nd week (p < 0.05) and the 4th week (p = 0.053), but not at the 8th week (Figure 5A). Principal component analysis revealed that gut microbiota in HFD group deviated from NC structure. At the 8th week, the gut bacterial composition profile in HFD+SC06 changed toward control group (Supplementary Figure S2). Histograms illustrating the gut microbiota structure revealed the microbial species and their relative abundance (Figure 6).
As obesity is characterized by a decrease in Bacteroidetes and an increase of Firmicutes (Nicholson et al., 2012). In the present study, we first determined the ratio of Firmicutes/Bacteroidetes. At the 4th week, HFD was associated with a significant increase in Firmicutes/Bacteroidetes (p < 0.05). Moreover, HFD+SC06 lowered Firmicutes/Bacteroidetes obviously compared to HFD-fed mice (Figure 6A). At phylum level, we also noticed that HFD increased Proteobacteria and TM7, especially at the 4th week (p < 0.05), while HFD+SC06 reduced TM7 abundance at the 4th week (p = 0.06). On the contrary, Verrucomicrobia almost disappeared in both HFD and HFD+SC06 groups (Figures 6B,C and Supplementary Figure S3). At family level, we found that S24-7 family accounted for the majority. At the 4th and 8th weeks, Clostridiaceae relative abundance was increased by HFD but inhibited by HFD+SC06 (p < 0.05) (Figure 6C and Supplementary Figure S3B). Furthermore, although most genera were unclassified, we also found that the genera profiles in HFD groups were distinct from that of NC groups. It is worth noting that increased Prevotella in HFD group was inhibited by HFD+SC06 at the 2nd week (p < 0.05) (Figure 6D and Supplementary Figure S3C). To compare community characteristics in more detail, heatmaps were constructed representing the relative abundance of species (Supplementary Figure S4). Results showed that most members of the gut microbiota belonging to S24-7 genus. The altered species in HFD group were seldom returned to normal by SC06 treatment at the 2nd and 4th weeks. However, some of the decreased S24-7 species (S24-7_346870, 315430, 264734, etc.) in mice receiving HFD were reversed in HFD+SC06 group at the 8th week.

LeFSe depicted time course changes of the dominant microbiota among groups (Figures 6E–G). We observed that at the 2nd week, the predominant intestinal flora in HFD were Bacteroidia and Betaproteobacteria. However, as time went on, branches of Bacilli became the major ones in HFD group at both 4th and 8th weeks. With HFD+SC06 feeding for different time points, the branches of Desulfovibrio, S24-7 and F16 were identified as novel superior microbiota (Figure 6).

**DISCUSSION**

Consistent with previous research (Xin et al., 2014), here we found that using the same HFD also successfully induced mice obesity and the related metabolic disorder, indicated by higher fat deposition, insulin resistance and hepatic steatosis. However, SC06 treatment effectively improved these features of metabolic syndrome. Coelomate animals coevolve with a diverse range of gut microbiota (Nicholson et al., 2012). The coevolution between host and gut microorganisms have led to a mutualistic relationship in which the microbiota contributes to many host physiological processes and in turn, hosts provide
When the mutualistic relationship is disrupted, the disordered gut microbiota leads to diseases (Littman and Pamer, 2011). Obesity and associated metabolic disorders are characterized by “low-grade” inflammation (Hotamisligil, 2006). Changes in gut microbiota and epithelial functions may play a crucial role in inflammation associated with obesity (Cani et al., 2008).

In this study, with the reduction of obesity, HFD+SC06 also significantly decreased the concentration of IL-6 and TNF-α compared to that of HFD. Thus, the role of SC06 in improving obesity and inflammation promoted us to figure out whether SC06 also attenuated gut bacterial dysbiosis. In mouse, the major distal gut microbiota are members belonging to Bacteroidetes and Firmicutes (Ley et al., 2005). Many studies have shown that the increased ratio of Firmicutes and Bacteroidetes is associated with the obesity phenotype (Ley et al., 2005; Moschen et al., 2012). In agreement with these findings, we observed that compared to NC group, Firmicutes/Bacteroidetes in HFD increased about twofold, which was decreased efficiently by SC06 administration. We also noticed that HFD increased relative abundance of TM7 and Proteobacteria at the 4th week, but decreased Verrucomicrobia and enriched TM7 was suppressed by SC06. In a previous report, obese individuals contained lower Verrucomicrobia abundance as well (Clarke et al., 2012). It was also suggested that Nlrp6-deficient mice with overrepresentation of Prevotellaceae and TM7 increased susceptibility to liver injury when on a methionine-choline-deficient to induce NASH (Elinav et al., 2011). TM7 can induce changes that result in inflammation in the colon and adipose tissues as well (Henao-Mejia et al., 2012). Differences were found not only at phyla level but also at all lower taxa levels among groups. Although it is shown that Clostridiales families were lowered in diet-induced obese mice (Clarke et al., 2013), a recent study found that Clostridiales was enriched in

FIGURE 5 | Changes in a-diversity of gut microbiota communities, as measured by (A) Simpson’s, (B) Goods_coverage’s, (C) Chao1’s, and (D) Observed_species’ Index Calculator. Differences between groups were determined by one-way ANOVA followed by Tukey’s t-test (n = 5). Significant differences between HFD versus NC are indicated as *p < 0.05, **p < 0.01. Significant differences between HFD versus HFD+SC06 are indicated as #p < 0.05.
FIGURE 6 | SC06 administration changed gut microbiota. (A) The ratio between Firmicutes and Bacteroidetes of gut microbiota, (B) phyla, (C) families, (D) genus of mice. Cladogram plotted from LeFSe analysis showing the taxonomic levels represented by rings with phyla in the outermost ring and genera in the innermost ring. Each circle is a member within that level. (E) Week 2, (F) week 4, and (G) week 8.

NAFLD patients (Jiang W.W. et al., 2015). Here, we found the increased Clostridiales induced by HFD was suppressed by SC06 treatment. Additionally, we also identified bacterial species whose abundance were altered by HFD and barely returned to control levels with SC06 approaches at the 2nd and 4th weeks. However, the abundance of species belonging to S24-7, including S24-7_346870, 315430 and 264734, was reversed by probiotic treatment at the 8th week. Bacteroidales S24-7 is a butyrate-producing bacterium associated with intestinal epithelial cell health (Villanueva-Millan et al., 2015). Qiu et al. (2017) believed S24-7 is negatively correlated with trimethylamine N-oxide, which can enhance accumulation of macrophage cholesterol and foam cell formation. On the contrary, Wu et al. (2017) considered S24-7 as the bad gut microbiota. Based on our results, we speculate that the increase of specific S24-7 strains might exert beneficial effect on the host. However, we didn’t notice significant differences for S24-7 abundance at family level between HFD and HFD+SC06 groups.
Because of the anatomical position and its unique vascular system, the liver is susceptible to the microbial products from the gut (Seo and Shah, 2012). Derangement of the gut microbiota occurs in a large percentage of patients with chronic liver disease, including NAFLD and NASH (Compare et al., 2012). The close interplay exists between the gut and liver is named “gut-liver axis.” Here, we also examined the liver injury in obese mice. HFD obviously increased GPT activity in the serum and elevated lipid accumulation, apoptosis and mitochondrial injury in the liver, which were reversed effectively in HFD + SC06. Lipid peroxidation and secondary cellular injury are the dominant mechanism in the transition from relatively stable hepatic steatosis to potentially progressive steatohepatitis in NAFLD. Oxidation of excessive fatty acids generates ROS that damage organelles and stimulate signaling pathways leading to fibrosis and cellular injury (Chang et al., 2006). Through analyzing the hepatic oxidation, we found that NC + SC06 significantly increased liver CAT activity. What’s more, oxidative stress was induced by HFD, but inhibited by SC06 administration, which was represented by the decreased hepatic ROS level and MDA concentration. Moreover, Nrf2 is a transcriptional activator that regulates cytoprotective gene expressions (Ishii et al., 2000). During oxidative stress, Nrf2 is released from its cytosolic repressor Keap1. The phosphorylated Nrf2 translocates into the nucleus where it binds to antioxidant reaction element residing within the promoter regions of many antioxidant and phase II genes (Lee and Johnson, 2004). According to our results, NC + SC06 elevated Nrf2 level to enhance the antioxidant ability. As a stress factor, HFD also significantly increased the phosphorylation of Nrf2 to resist the oxidative stress induced by diet. In the previous study, compounds with antioxidant capacity could activate Nrf2 pathway in combination with HFD (Feng et al., 2017). However, in the present study, with HFD + SC06 treatment, p-Nrf2 expression was markedly decreased. As far as we concerned, since the ROS and MDA levels were reduced in HFD + SC06 treated mice, it is not necessary for hepatocytes to activate more Nrf2 and increase antioxidase activities to combat with oxidative stress. Additionally, recent studies demonstrated Nrf2 ablation in mice adipose tissue prevented diet-induced obesity (Zhang et al., 2016), and mice lacking Nrf2 had more energy expenditure and resisted to diet-induced obesity (Schneider et al., 2016). Thus, as far as we concerned, the obvious inactivation of Nrf2 induced by HFD + SC06 might also protect against HFD-induced lipid accumulation.

Epithelial function is important for the development of NAFLD and obesity (Raybould, 2012). In a previous research, we suggested that SC06 was able to elevate the oxidation resistant capacity of intestinal porcine epithelial cell line (Wang et al., 2017), and the present study has provided novel information regarding the effects of SC06 on gut microbiota, hepatic steatosis and oxidative stress in mice (Figure 7).

Taken together, we propose that SC06 protected mice from HFD-induced obesity and liver injury through (1) directly improving the epithelial function by enhancing intestinal epithelial cell antioxidant capacity, (2) decreasing inflammation, lowering hepatic oxidative damage and (3) regulating gut microbiota composition, especially Bacteroides, Firmicutes, TM7 phyla and species belonging to S24-7. These findings may aid in the application of probiotic Bacillus in the food industry to improve human and animal’s health. However, further investigation about the correlations among the decreased obesity, oxidative stress and gut bacterial composition are needed.
AUTHOR CONTRIBUTIONS

WL and YPW designed the experiments. YPW, VX, and XX performed the animal husbandry. BW, XZ, and JN analyzed the 16S rRNA data. YW and XM did the animal experiments. YW wrote the final manuscript. All authors approved and contributed to the final version of the manuscript.

FUNDING

This study was supported by the National Natural Science Foundation of China (Nos. 31672460 and 31472128), the National High-Tech R&D Program (863) of China (No. 2013AA102803D), the Major Science and Technology Project of Zhejiang Province (No. 2006C12086), and the Key Laboratory of Animal Feed and Nutrition of Zhejiang Province (No. 201802).

REFERENCES

Backhed, F., Ding, H., Wang, T., Hooper, L. V., Koh, G. Y., Nagy, A., et al. (2004). The gut microbiota as an environmental factor that regulates fat storage. Proc. Natl. Acad. Sci. U.S.A. 101, 15718–15723. doi: 10.1073/pnas.0407076101

Birse, R. T., Choi, J., Reardon, K., Rodriguez, J., Graham, S., Diop, S., et al. (2010). High-fat-diet-induced obesity and heart dysfunction are regulated by the TOR pathway in Drosophila. Cell. Metab. 12, 533–544. doi: 10.1016/j.cmet.2010.09.014

Cani, P. D., Bibiloni, R., Knauf, C., Neyrinck, A. M., Delzenne, N. M., et al. (2008). Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. Diabetes 57, 1470–1481. doi: 10.2337/db07-1403

Carmiel-Haggai, M., Cederbaum, A. I., and Nieto, N. (2004). A high-fat diet leads to increased oxidative stress and heart dysfunction in mice. Toxicol. Appl. Pharmacol. 195, 252–264. doi: 10.1016/j.taap.2004.03.023

Chang, C. Y., Argo, C. K., Al-Osaimi, A. M., and Caldwell, S. H. (2006). Therapy of NAFLD: antioxidants and cytoprotective agents. J. Clin. Gastroenterol. 40(Suppl. 1), S51–S60.

Charradi, K., Elkahoui, S., Limam, F., and Aouani, E. (2013). High-fat diet induced colitis. J. Apoptosis 8:e5790. doi: 10.1371/journal.pone.0065790

Compare, D., Coccoli, P., Rocco, A., Nardone, O. M., De Maria, S., Carteni, M., et al. (2012). Gut-liver axis: the impact of gut microbiota on non-alcoholic fatty liver disease. Nutr. Metab. Cardiovasc. Dis. 22, 471–476. doi: 10.1016/j.numecd.2012.02.007

Dietz, W. H., Baur, L. A., Hall, K., Puhl, R. M., Taveras, E. M., Uauy, R., et al. (2015). Management of obesity: improvement of health-care training and systems for prevention and care. Lancet 385, 2521–2533. doi: 10.1016/S0140-6736(14)61748-7

Do, H. J., Chung, J. H., Hwang, J. W., Kim, O. Y., Lee, J. Y., and Shin, M. J. (2015). 1-Deoxynojirimycin isolated from Bacillus subtilis improves hepatic lipid metabolism and mitochondrial function in high-fat-fed mice. Food Chem. Toxicol. 75, 1–7. doi: 10.1016/j.fct.2014.11.001

Elinav, E., Strovig, T., Kau, A. L., Henao-Mejia, J., Thaiss, C. A., Booth, C. J., et al. (2011). NLRP6 inflammasome regulates colonic microbial ecology and risk for colitis. Cell 145, 745–757. doi: 10.1016/j.cell.2011.04.022

Feng, X. J., Yu, W., Li, X. D., Zhou, F. F., Zhang, W. L., Shen, Q., et al. (2017). Apigenin, a modulator of PPAR gamma, attenuates HFD-induced NAFLD by regulating hepatocyte lipid metabolism and oxidative stress via Nrf2 activation. Biochem. Pharmacol. 136, 136–149. doi: 10.1016/j.bcp.2017.04.014

Foster, T., Budoff, M. J., Saab, S., Ahmed, N., Gordon, C., and Guerci, A. D. (2011). Atorvastatin and antioxidants for the treatment of nonalcoholic fatty liver disease: the St Francis heart study randomized clinical trial. Am. J. Gastroenterol. 106, 71–77. doi: 10.1038/ajg.2010.299

Furukawa, S., Fujita, T., Shimabukuro, M., Iwaki, M., Yamada, Y., Nakajima, Y., et al. (2004). Increased oxidative stress in obesity and its impact on metabolic syndrome. J. Clin. Invest. 114, 1752–1761. doi: 10.1172/JCI21625

Hallen, J. E., de Carmo, J. M., da Silva, A. A., Wang, Z., and Hallen, M. E. (2015). Obesity-induced hypertension interaction of neurohumoral and renal mechanisms. Circ. Res. 116, 991–1006. doi: 10.1161/Circresaha.116.305697

Haslam, D. W., and James, W. P. T. (2005). Obesity. Lancet 366, 1197–1209. doi: 10.1016/S0140-6736(05)67483-1

Henoa-Mejia, J., Elinav, E., Jin, C., Hao, L., Mehal, W. Z., Strovig, T., et al. (2012). Inflammasome-mediated dysbiosis regulates progression of NAFLD and obesity. Nature 482, 178–185. doi: 10.1038/nature10809

Hotamisligil, G. S. (2006). Inflammation and metabolic disorders. Nature 444, 860–867. doi: 10.1038/nature05485

Ishii, T., Itoh, K., Takahashi, S., Sato, H., Yanagawa, T., Katoh, Y., et al. (2000). Transcripton factor Nf2 coordinates a group of oxidative stress-inducible genes in macrophages. J. Biol. Chem. 275, 16023–16029. doi: 10.1074/jbc.275.21.16023

Jiang, W. W., Wu, N., Wang, X. M., Chi, Y. J., Zhang, Y. Q., Qiu, X. Y., et al. (2015). Dysbiosis gut microbiota associated with inflammation and impaired mucosal immune function in intestine of mice with non-alcoholic fatty liver disease. Sci. Rep. 5:8096. doi: 10.1038/srep08096

Jiang, Y., Xu, Y., Bi, Y. F., Wang, L. M., Zhang, M., Zhou, M. G., et al. (2015). Prevalence and trends in overweight and obesity among Chinese adults in 2004-10: data from three nationwide surveys in China. Lancet 386:77. doi: 10.1016/S0140-6736(15)00658-3

Jones, R. M., Desai, C., Darby, T. M., Luo, L., Wolfarth, A. A., Scharer, C. D., et al. (2015). Lactobacillus modulate epithelial cytoprotection through the Nrf2 Pathway. Cell Rep. 12, 1217–1225. doi: 10.1016/j.celrep.2015.07.042

Jumperza, R., Le, D. S., Turnbaugh, P. J., Trinidad, C., Bogardus, C., Gordon, J. I., et al. (2011). Energy-balance studies reveal associations between gut microbes, caloric load, and nutrient absorption in humans. Am. J. Clin. Nutr. 94, 58–65. doi: 10.3945/ajcn.110.10132

Kamada, N., Seo, S. U., Chen, G. Y., and Nunez, G. (2013). Role of the gut microbiota in immunity and inflammatory disease. Nat. Rev. Immunol. 13, 321–335. doi: 10.1038/nri3430

Kim, S., Sohn, I., Ahn, J. I., Lee, K. H., Lee, Y. S., and Lee, Y. S. (2004). Hepatic gene expression profiles in a long-term high-fat-diet-induced obesity mouse model. Gene 340, 99–109. doi: 10.1016/j.gene.2004.06.015

Kirpich, I. A., Marsano, L. S., and McClain, C. J. (2015). Gut-liver axis, nutrition, and non-alcoholic fatty liver disease. Clin. Biochem. 48, 923–930. doi: 10.1016/j.clinbiochem.2015.06.023

Kopelman, P. G. (2000). Obesity as a medical problem. Nature 404, 635–643. doi: 10.1038/35075308

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2019.01161/full#supplementary-material
Kumar, M., Nagpal, R., Kumar, R., Hemalatha, R., Verma, V., Kumar, A., et al. (2012). Cholesterol-lowering probiotics as potential biotherapeutics for metabolic diseases. *Exp. Diabetes Res.* 2012:902917. doi: 10.1155/2012/902917

Lee, J. M., and Johnson, J. A. (2004). An important role of Nrf2-ARE pathway in the cellular defense function. *J. Biochem. Mol. Biol.* 37, 139–143. doi: 10.5483/bmbrep.2004.37.2.139

Lei, K., Li, Y. L., Wang, Y., Wen, J., Wu, H. Z., Yu, D. Y., et al. (2015). Effect of dietary supplementation of *Bacillus subtilis* B10 on biochemical and molecular parameters in the serum and liver of high-fat diet-induced obese mice. *J. Zhejiang Univ. Sci. B* 16, 487–495. doi: 10.1631/jzus.B1400342

Ley, R. E., Backhed, F., Turnbaugh, P., Lozupone, C. A., Knight, R. D., and Gordon, J. I. (2005). Obesity alters gut microbial ecology. *Proc. Natl. Acad. Sci. U.S.A.* 102, 11070–11075. doi: 10.1073/pnas.0504978102

Li, C., Nie, S. P., Zhu, K. X., Ding, Q., Li, C., Xiong, T., et al. (2014). *Lactobacillus plantarum* NCU116 improves liver function, oxidative stress and lipid metabolism in rats with high fat diet induced non-alcoholic fatty liver disease. *Food Funct.* 5, 3216–3223. doi: 10.1039/c4fo00549j

Littman, D. R., and Pamer, E. G. (2011). Role of the commensal microbiota in normal and pathogenic host immune responses. *Cell Host Microbe* 10, 311–323. doi: 10.1016/j.chom.2011.10.004

Moschen, A. R., Wieser, V., and Tilg, H. (2012). Dietary factors: major regulators of the gut’s microbiota. *Gut Liver* 6, 411–416. doi: 10.5009/gnl.2012.6.4.411

Musso, G., Anty, R., and Petta, S. (2013). Antioxidant therapy and drugs interfering with lipid metabolism: could they be effective in NAFLD patients? *Curr. Pharm. Design.* 19, 5297–5313. doi: 10.2174/1381612811319290010

Nicholson, J. K., Holmes, E., Kinross, J., Bercelin, R., Gibson, G., Jia, W., et al. (2012). Host-gut microbiota metabolic interactions. *Science* 336, 1262–1267. doi: 10.1016/j.science.2012.08.015

Nikoskelainen, S., Ouwehand, A. C., Bylund, G., Salminen, S., and Lilius, E. M. (2003). Immune enhancement in rainbow trout (*Oncorhynchus mykiss*) by potential probiotic bacteria (*Lactobacillus rhamnosus*). *Fish Shellfish Immunol.* 15, 443–452. doi: 10.1016/S0927-6585(02)00115-4

Ogden, C. L., Carroll, M. D., and Flegal, K. M. (2014). Prevalence of obesity in the United States. *JAMA J. Am. Med. Assoc.* 312:189. doi: 10.1001/jama.2014.6219

Park, S., Ji, Y., Jung, H. Y., Park, H., Kang, J., Choi, S. H., et al. (2017). *Lactobacillus plantarum* HAC01 regulates gut microbiota and adipose tissue accumulation in a diet-induced obesity murine model. *Appl. Microbiol. Biotechnol.* 101, 1605–1614. doi: 10.1007/s00253-016-7959-2

Qiu, L., Yang, D., Tao, X. Y., Yu, J., Xiong, H., and Wei, H. (2017). *Enterobacter aerogenes* ZDY01 attenuates choline-induced trimethylamine N-Oxide levels by remodeling gut microbiota in mice. *J. Microbiol. Biotechnol.* 27, 1491–1499. doi: 10.4041/jmb.1703.03039

Rajput, I. R., Li, W. F., Li, Y. L., Jian, L., and Wang, M. Q. (2013). Application of probiotic (*Bacillus subtilis*) to enhance immunity, antioxidation, digestive enzymes activity and hematological profile of Sockeye Duck. *Pak. J. Vet. J.* 33, 69–72.

Raybould, H. E. (2012). Gut microbiota, epithelial function and derangements in obesity. *J. Physiol.* 590, 441–446. doi: 10.1113/jphysiol.2011.222133

Schneider, K., Valdez, J., Nguyen, J., Vawter, M., Galke, B., Kurtz, T. W., et al. (2016). Increased energy expenditure, Ucp1 expression, and resistance to diet-induced obesity in mice lacking nuclear factor-erythroid-2-related transcription factor-2 (Nrf2). *J. Biol. Chem.* 291, 7754–7766. doi: 10.1074/jbc.m115.673756

Seo, Y. S., and Shah, V. H. (2012). The role of gut-liver axis in the pathogenesis of liver cirrhosis and portal hypertension. *Clin. Mol. Hepatol.* 18, 337–346. doi: 10.3350/chm2012.18.4.337

Stolz, D. B., Ross, M. A., Salem, H. M., Mars, W. M., Michalopoulos, G. K., and Enomoto, K. (1999). Cationic colloidal silica membrane perturbation as a means of examining changes at the sinusoidal surface during liver regeneration. *Am. J. Pathol.* 155, 1487–1498. doi: 10.1016/s0002-9440(10)65464-8

Tappy, L., and Le, K. A. (2010). Metabolic effects of fructose and the worldwide increase in obesity. *Physiol. Rev.* 90, 23–46. doi: 10.1152/physrev.00019.2009

Turnbaugh, P. J., Baeckhed, F., Fulton, L., and Gordon, J. I. (2008). Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. *Cell Host Microbe* 3, 213–223. doi: 10.1016/j.chom.2008.02.015

Villanueva-Millan, M. J., Peretz-Matute, P., and Oteo, J. A. (2015). Gut microbiota: a key player in health and disease. A review focused on obesity. *J. Physiol. Biochem.* 71, 509–525. doi: 10.1016/j.sjpbi.2015.03.090-3

Wang, J. J., Tang, H., Zhang, C. H., Zhao, Y. F., Derrien, M., Rocher, E., et al. (2015). Modulation of gut microbiota during probiotic-mediated attenuation of metabolic syndrome in high fat diet-fed mice. *ISME J.* 9, 1–15. doi: 10.1038/ismej.2014.99

Wang, Y., Wu, Y. P., Wang, Y. B., Fu, A. K., Gong, L., Li, W. F., et al. (2017). *Bacillus amyloliquefaciens* SC06 alleviates the oxidative stress of IPEC-J2 cell modulating Nrf2/Keap1 signaling pathway and decreasing ROS production. *Appl. Microbiol. Biotechnol.* 101, 3015–3026. doi: 10.1007/s00253-016-8832-4

Wu, Q. Z., Zhang, H. C., Wang, P. G., and Chen, M. (2017). Evaluation of the efficacy and safety of *Ganoderma lucidum* mycelium-fermented liquid on gut microbiota and its impact on cardiovascular risk factors in human. *Rsc. Adv.* 7, 45093–45100. doi: 10.1039/c7ra08087e

Xin, J. G., Zeng, D., Wang, H. S., Ni, X. Q., Yi, D., Pan, K. C., et al. (2014). Jing. B. Preventing non-alcoholic fatty liver disease through *Lactobacillus johnsonii* BS15 by attenuating inflammation and mitochondrial injury and improving gut environment in obese mice. *Appl. Microbiol. Biotechnol.* 98, 6817–6829. doi: 10.1007/s00253-014-7572-1

Zhang, L., Dasuri, K., Fernandez-Kim, S. O., Bruce-Keller, A. J., and Keller, J. N. (2017). Metabolic effects and pathogenicity of *Pseudomonas aeruginosa* strain Nrf2 studied by RNA-Seq analysis. *J. Biol. Chem.* 292, 6737–6747.

**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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