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Whole grain oat attenuates high-fat and high-cholesterol diet-induced dyslipidemia by modulating non-12OH bile acid ratio via the gut-liver axis

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ABSTRACT: Background: Dyslipidemia is a chronic, metabolic disease characterized by elevated level of serum total cholesterol (TC) or low-density lipoprotein cholesterol (LDL-C), and acts as a key contributor to the development of cardiovascular diseases (CVDs). Our previous work has shown that whole grain oat supplementation exerted cholesterol-lowering properties by modulating gut microbiota in individuals with hyperlipidemia. However, it is not clear that whether the cholesterol-lowering effect of whole grain oat depends on gut microbiota and its metabolites. In this study, we leverage a high-fat and high-cholesterol (HFHC) diet induced model of dyslipidemia that exhibits significant remission following whole grain oat intervention, to conduct an animal experiment that integrates clinical parameters, 16S rRNA sequencing, targeted metabolomic profiling and fecal microbiota transplantation (FMT) to investigate the relationship between whole grain oat diet, gut microbiota, and dyslipidemia.

Results: We demonstrated that the improvements in blood circulating TC and LDL-C level induced by a whole grain oat diet is accompanied by alterations in gut microbiota diversity and structure marked by increased abundance of probiotics (e.g., *Bifidobacterium* and *Parabacteroides*). Targeted metabolomic profiling analysis showed that whole grain oat supplementation increases the ratio of non-12OH/12-OH bile acid (BA), level of hyodeoxycholic acid (HDCA), glycochenodeoxycholic acid-3S (GCDCA-3S), isodeoxycholic acid (IDCA), and propionate acid. Correlation analysis showed that the alteration in the metabolic profiles is closely related to the changed microbial taxa. Consistently, the expression level of proteins involved in BAs synthesis (especially the alternative pathway) was significantly activated by whole grain oat supplementation. Subsequently, FMT treatment attenuated the abnormal increase of serum lipid in the model rat. Finally, when HDCA was administered to rats for 8 weeks, the circulating TC and LDL-C level were significantly ameliorated.

Conclusions: These data revealed that whole grain oat supplementation ameliorated the dyslipidemia in a gut microbiota-dependent manner, via promoting the production of short-chain fatty acids (SCFAs), activating the alternative pathway of BAs metabolism and increase the ratio of non-12OH/12-OH BAs. Our findings provide a promising, and novel therapeutic strategy for gut microbiota to prevent and treat dyslipidemia.

Key words: whole grain oat, dyslipidemia, gut microbiota, BAs metabolism, gut-liver axis

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1. Introduction

Dyslipidemia, with a sharply increased prevalence over the past 30 years, is a group of metabolic derangements characterized by the alterations of plasma lipid profile, including elevated low-density lipoprotein cholesterol (LDL-C), elevated total cholesterol (TC), elevated triglycerides (TG) and (or) reduced high-density lipoprotein cholesterol (HDL-C) [1]. Compelling evidence have indicated that lipid abnormalities can result in the progressive accumulation of cholesterol in arterial walls, and significantly increase risk of cardiovascular diseases (CVDs) [2, 3]. The fact that CVDs are the leading cause of mortality worldwide, it is reported that approximately 18 million lives were taken by CVD-related illnesses each year [4, 5]. In China, CVDs such as stoke and ischemic heart disease have become the leading contributor to death and disability-adjusted life-years (DALYs) [6]. Clinically, endogenous cholesterol synthesis is one of crucial pharmacological targets for cholesterol-lowering drugs such as statin. However, clinicians should consider the possible adverse effects taken by different drug treatments [7]. In this situation, there is an unmet need for developing new therapeutic strategies for the primary prevention of dyslipidemia.

Although a range of non-modifiable risk factor such as family gene and age have been presented to promote the occurrence of dyslipidemia, most of noncommunicable diseases could be largely prevented through diet and nutrition therapy [8, 9], with perhaps the clearest example of this being the recommendation of health behavior modifications as primary prevention for CVDs proposed by Canadian Cardiovascular Society [10]. Consistently, numerous epidemiological studies and meta-analyses have shown that whole grain consumption is negatively correlated with the occurrence of CVDs [11-13]. Previous clinical trials done in our lab suggested that patients with mild dyslipidemia can gain benefits from whole grain oat consumption on lowering serum cholesterol by modulating gut microbiota and short-chain fatty acids (SCFAs) [14, 15]. Also, rodent experiments have provided us some mechanistic clues involving gut microbiota [16-18]. But whether the gut microbiota and its metabolites play a dependent role in whole grain oat-mediated improvements of dyslipidemia remain largely controversial.

The gut microbiota, which acts as an important factor affecting host physiology, is a community of over trillions of bacteria that reside in human intestine. Previous studies showed that individuals with reduced microbial gene richness usually present a more pronounced dys-metabolism in lipids, and all of these unfavorable biomarkers could be improved by dietary intervention [19, 20]. An increased abundance of *Lactobacillus* and *Bifidobacterium* can ameliorate the development of dyslipidemia in both dietary models in mice and clinical trials [21, 22]. It is reported that these genera can promote de novo synthesis of bile acid through serving cholesterol as substrate, and lead to more excretion of secondary bile acids from feces [23, 24]. In contrast, dysbiosis also exerts a tremendous impact on dyslipidemia. For instance, in a validation cohort study conducted by Zeng et al., they demonstrated that *[Ruminococcus] gnavus, Alistipes shahii,* and *Lachnospira eligens* mediated the associations between lifestyles and risks of dyslipidemia, with potential metabolic pathways involving arachidonic acid, bile acid, and aromatic amino-acid metabolism [25]. Similar mechanisms were further supported by Zhong et al., who found that the deficiency of *Family_XIII_UCG-001*

genus may inhibit expression of lipid metabolism pathways, including MAPK and Rap1 signaling pathway, thereby aggravating lipid metabolism disorders [26]. Moreover, findings from the LifeLines-DEEP population cohort suggested that the variance of 6% in TG and 4% in HDL-C can be explained by human gut microbiota composition [27]. These results furtherly highlight a potential role for gut microbiota in metabolic diseases.

Bile acids (BAs) and SCFAs are the dominant products derived from the co-metabolism of host and gut microbiota [28-30]. BAs are synthesized from cholesterol in the liver through classic and alternative pathways, and the end-products can be classified as 12OH BAs (mainly from classic pathway including cholic acid (CA), deoxycholic acid (DCA) and their conjugates) and non-12OH BAs (mainly from alternative pathway including chenodeoxycholic acid (CDCA), lithocholic acid (LCA) and their conjugates), respectively [31]. It has been proposed that dietary fiber can significantly increase CDCA and LCA in cholesterol-fed rats [32]. Further, recent study demonstrated that activation of alternative pathway can improve the status of glucolipid metabolism in body. For example, Huang et al. found that theabrownin from Pu-erh tea can attenuates hypercholesterolemia via increasing the gene expression involving in the BA alternative pathway accompanied by the inhibition of intestinal farnesoid X receptor/fibroblast growth factor 15 (FXR/FGF15) signaling pathway [33]. On the other hand, SCFAs are primary end-products of fermentation of non-digestible carbohydrates (such as oat β-glucan) that become available to the gut microbiota. Accumulating studies have found that SCFAs can participant in amounts of physiological processes, including lipid metabolism and energy homeostasis [34, 35]. Haghikia et al. demonstrated that propionic acid supplementation significantly reduced intestinal cholesterol absorption and aortic atherosclerotic lesion area in apolipoprotein $E^{-/-}$ mice, then similar findings were also observed in a clinical trial performed in patients with elevated LDL-C [36]. Therefore, targeting the microbiota-derived metabolites might be a promising approach for preventing and attenuating dyslipidemia.

Despite the fact that the gut microbiota and its metabolites have been implicated in dyslipidemia pathogenesis and that healthy diet (such as whole grain diet) can be used to manage symptoms of dyslipidemia, there is limited insight into how this occurs. In this study, we hypothesized that the gut microbiota modulated by whole grain oat alters the BAs profiles in the gut, leading to an enhanced level of cholesterol metabolism in liver. To this end, we evaluated the favorable effect of whole grain oat on lipid profiles in a high-fat and high-cholesterol (HFHC) diet induced hyperlipidemic rat model. Furthermore, high-throughput sequencing, targeted metabolomics and molecular biology technology were employed to explore the underlying mechanisms involved. Finally, the dependent role of gut microbiota and its metabolites were examined through fecal microbiota transplantation (FMT) and oral administration experiment. These studies will offer new theoretical basis on the improvement of the gut microbiota and BAs profiles upon whole grain oat supplementation, which will provide new approaches to the treatment and prevention of dyslipidemia induced by unhealthy diet.

2. Results

2.1 Whole grain oat reduced blood circulating TC and LDL-C in high-fat and high cholesterol diet-induced dyslipidemia in rat

To study the alleviated effect of whole grain oat on high-fat diet-induced dyslipidemia, experimental dyslipidemia was induced in rat with a HFHC feed (Research diet: D12109C, containing 40% energy from fat and added 1.25% cholesterol). No significant between-group difference in body weight and food intake were found during the experiment (Fig. 1a, b). After 12-week HFHC intervention, the rat in model group have developed dyslipidemia, with increased level of serum TC, LDL-C and TG, and reduced level of serum HDL-C compared with normal group. Whole grain oat supplementation significantly decreased the level of serum TC and LDL-C, indicating the hypolipidemic effect of oat. Moreover, the effect seems to be more pronounced with the increase of intervention dosage (Fig. 1c).

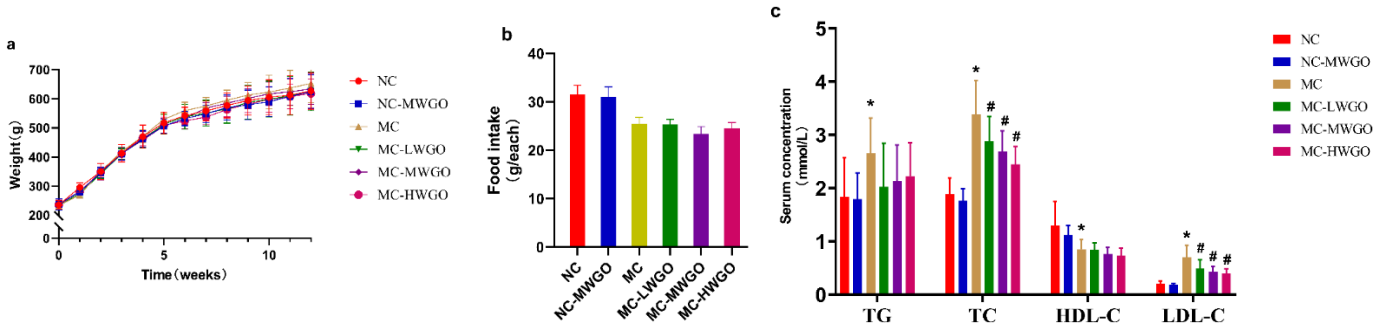

Fig 1. Body weight, food intake and lipid lowering effects of whole grain oat. **a** The effect of whole grain oat on body weight during 12-week HFHC intervention; **b** The daily food intake of normal diet or HFHC fed rats during 12 weeks of whole grain oat intervention; **c** Whole grain oat reduced serum TC and LDL-C of rats with HFHC diet for 12 weeks. Data were expressed as mean ± SD. Differences were analyzed by one-way analysis of variance (ANOVA) with post hoc tests for multiple group comparisons. $^{*}P < 0.05$ compared with NC group, $^{\#}P < 0.05$ compared with MC group, n=8 individuals/group.

2.2 Whole grain oat reshaped the structure and diversity of the gut microbiota

Fecal samples were harvested at the end of the experiment. We employed rarefaction curve, species accumulation curves and rank abundance curve to measure the quality of 16S rRNA sequencing, and the results showed that the data were reliable and could be further analyzed (Supplementary Fig. 2). In comparison with NC group, α-diversity (e.g., Chao 1, Observed species, Shannon and Simpson index) in MC group had a significant decrease, indicating a dysbiosis on the microbial community richness and diversity induced by HFHC diet, while Shannon index was reversed by MC-M/HWGO groups compared to MC group (Fig. 2a). Principal coordinates analysis (PCoA) based on weighted unifrac distance revealed that all interventional samples clustered closely and was more similar to that of the NC group while clustering away from vehicle-treated HFHC-fed rat after 12 weeks of whole grain oat supplementation, the first principal coordinate in the model could explain 39% of the overall variations (Fig. 2b). Hierarchical clustering analysis suggested that MC-HWGO group was in the same clustering branch with NC group (Fig. 2c), these significant findings were consistent with nonmetric multidimensional scaling (NMDS) results (Stress=0.0815, Fig. 2d). In terms of gut microbiota composition, HFHC diet led to increases in *Fimicutes* at phylum level and *Blautia* at genus level, while three oat intervention groups prevented HFHC-driven reduction in *Bacteroidetes* and *Lactobacillus* (Fig. 2e). Besides, univariate analysis method was used to

investigate the significant difference in microbial taxa between groups after 12 weeks of whole grain oat treatment. The results showed that HFHC-fed rat displayed significant reductions in the abundance of *Lactobacillus, Prevotella, Ruminococcaceae_Ruminococcus, Butyricimonas* and *Clostridiaceae_Clostridium,* but higher abundance of *Blautia* and *Lachnospirceae_Clostridium* (Fig. 2f). However, certain probiotics such as *Bifidobacterium* and *Parabacteroides* had notably increase under whole grain oat supplementation, while the expanded abundance of *Lachnospirceae_Clostridium* in HFHC-fed rat showed a significant decrease (Fig. 2g). These results revealed that whole grain oat can reshaped the gut dysbiosis induced by HFHC diet in favor of a healthy phenotype.

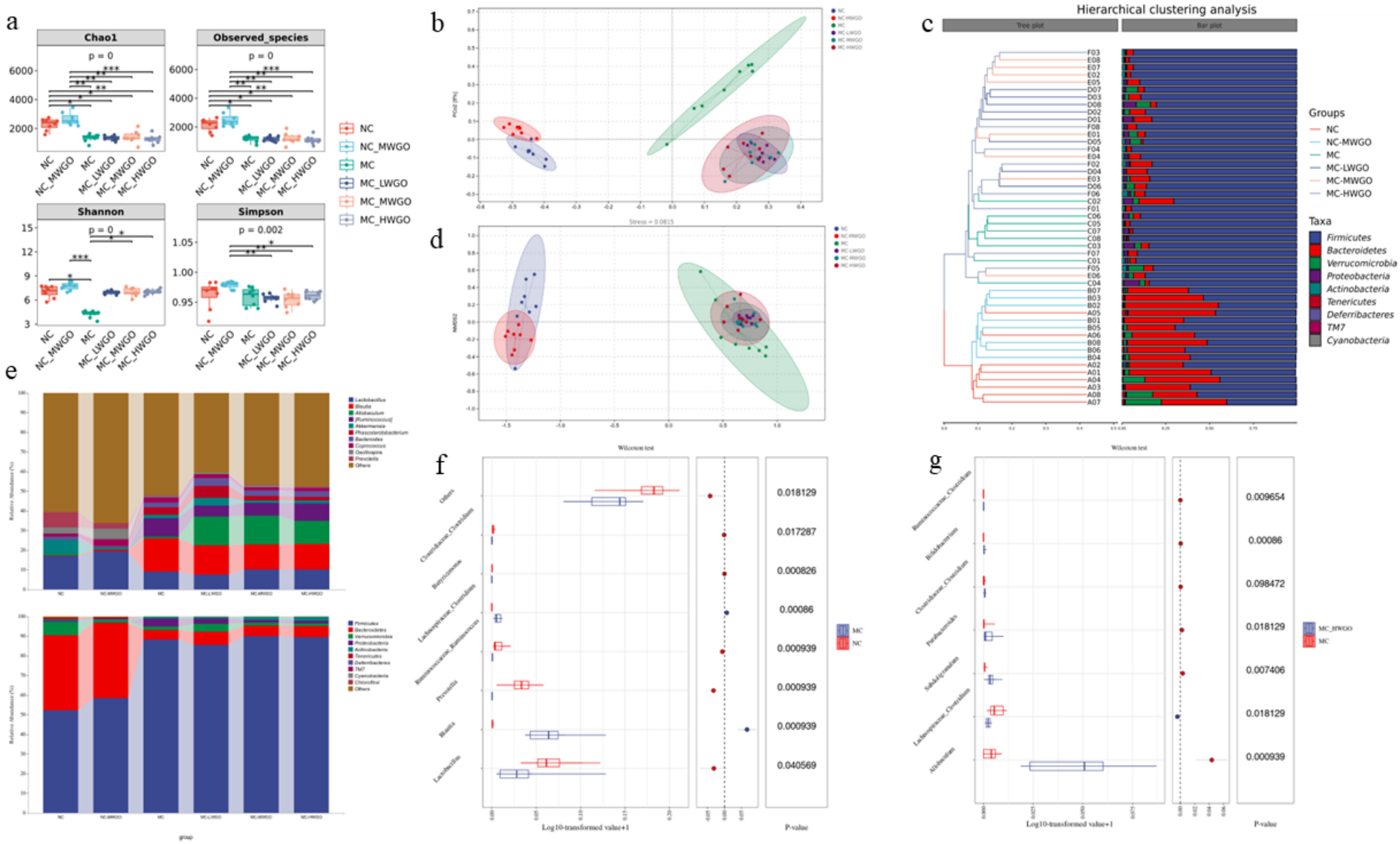

Fig 2. Whole grain oat improved the structure and diversity of gut microbiota in HFHC-fed rats. **a** The effect of whole grain oat on α-diversity index; **b** Principal coordinate analysis (PCoA) score plots based on unweighted unifrac distance; **c** Hierarchical clustering analysis based on phylum level. **d** NMDS analysis based on unweighted unifrac distance. **e** Relative abundance of top 10 phyla in each group (Top:NC vs MC; Bottom: MC vs MC-HWGO). **f** Significant difference in genus level between NC and MC group. g Significant difference in genus level between MC and MC-HWGO group. n=8 individuals/group.

2.3 FMT treatment alleviated the biochemical parameters in the model rat

In order to measure the effectiveness of antibiotics treatment in establishing germ-free rat model, we collected the fecal samples at the before and the end of 2-week antibiotics treatment, and performed 16S rRNA sequencing. The result showed that antibiotics treatment led to a remarkable reduction from 12080 to 770 in the number of operation taxonomic units (OTUs) (Supplementary Fig. 3a). Besides, the richness and diversity of microbial taxa existed in single sample had been almost destroyed by antibiotics treatment (Supplementary Fig. 3b). PCoA displayed a significant separation of the composition of gut microbiota (Supplementary Fig. 3c), there were only 3 genus taxa (*Providencia, Shigella,* and *Pseudomonadaceae_Pseudomonas*, Supplementary Fig. 3d) existed in the samples treated with antibiotics, this disruption was further confirmed by NMDS analysis (Stress=0.0792, Supplementary Fig. 3e). Above all, the germ-free model rat established in present study is suitable to accept the fecal microbiota from a donor. To examined whether the gut microbiota from donor colonized recipient's intestinal tract, we continued to explore the changes of gut microbiota in various groups. The results showed that the diversity in recipient was significant improved (Supplementary Fig. 4a, b), and the eliminated microbial taxa was restored and exhibited similar to their donors (Supplementary Fig. 4c, d). These results indicated that the gut microbiota from donor was successfully colonized in the recipient through FMT.

As expected, significant decreased level of serum TC and LDL-C were seen in the rat treated with the FMT from MC-HWGO compared with the control. Of note, the effect was not significantly different when oral administration of various dosage of fecal bacteria solution obtained from the same donor (Fig. 3b, c). However, we did not observed significant difference in the ratio of 12OH/NON 12OH BAs after FMT between the groups (Fig. 3a, P=0.057).

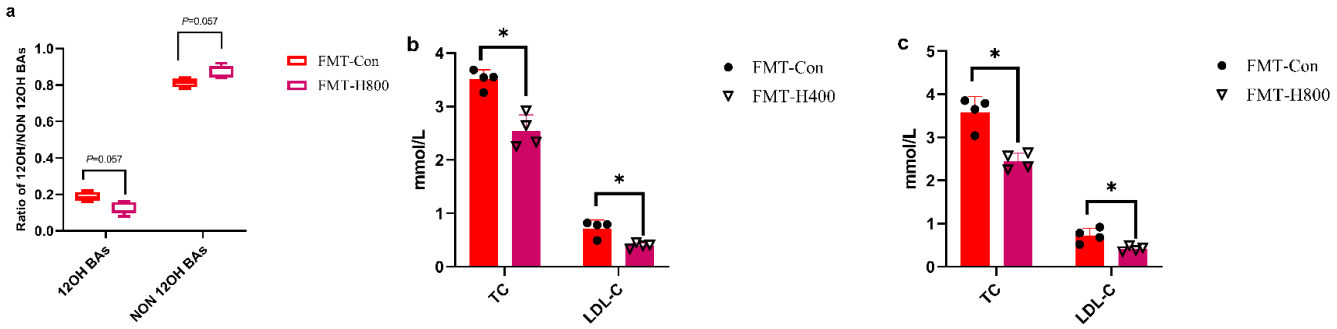

Fig 3. The effect of FMT on serum lipid. a The effect of FMT supplied with fecal suspension of 800μL from MC-HWGO on fecal BAs; **b** The effect of FMT supplied with fecal suspension of 400μL from MC-HWGO on serum lipid; **c** The effect of FMT supplied with fecal suspension of 800μL from MC-HWGO on serum lipid; FMT-Con: recipient group supplied with fecal suspension from MC; FMT-H400: recipient group supplied with fecal suspension of 400μL from MC-HWGO; FMT-H800: recipient group supplied with fecal suspension of 800μL from MC-HWGO, *P < 0.05 compared with control group, n=5 individuals/group.

2.4 Whole grain oat selectively altered the BAs and SCFAs profiles in the gut, and their correlations with gut microbiota

Since BAs metabolism is crucial for the maintenance of cholesterol homeostasis, we further evaluated whether shifts of BAs pool in the cecum could be associated with the effect presented in whole grain oat intervention. Supervised orthogonal partial least squares discriminant analysis (OPLS-DA) was applied to

visualize between-group difference. There was a significant separation between NC and MC group, and a total of 34 significantly changed BAs have been identified, indicating a potential disturbance caused by HFHC feeding (Supplementary Fig. 5). Compared to MC group, MC-HWGO group presented a good performance for separation in BAs profile from MC group (Fig. 4a, $Q^2X=0.785$, $R^2Y=0.983$). In addition, 9 differential BAs between the groups were identified furtherly with a variable-importance-in-projection (VIP) value of >1 combined with the fold change ≥ 2 or ≤ 0.5. Animals treated with high-dose oat showed significant reductions of 6 BAs (TCA, TDCA, DLCA, 12KLCA, 23-DCA, GDHCA), and increases of 3 BAs (HDCA, GCDCA-3S, IDCA) compared with MC group (Fig. 4b). Furthermore, we investigated the alteration in the ratio of various categories of bile acids (primary/secondary, unconjugated/conjugated, and 12OH/non-12OH) after interventions. HFHC diet obviously led to an increased level of primary, unconjugated and 12OH BAs, other 3 categories of BAs significantly decreased correspondingly (Supplementary Fig. 6). However, high-dose oat treatment significantly reduced the ratio of 12OH BAs and increased non-12OH BAs ratio, while no significant effect was found in the ratio of primary/secondary and unconjugated/conjugated BAs (Fig. 4c). These findings indicated that HFHC diet can induce a redistribution of the ratio of all categories of BAs, among which a high ratio of 12OH to non-12OH may cause adverse effect on BA homeostasis.

We next focused on the effect of whole grain oat on SCFAs, who act as important end productions of non-digestible dietary fiber fermentation by gut microbiota. All 7 SCFAs were significantly diminished by HFHC diet, but compared to MC group, our assessment uncovered that only the production of propionate acid was significantly improved by MC-HWGO group (Fig. 4d).

We used Spearman correlation analysis to further evaluate the association between differentially enriched microbial taxa and BAs or SCFAs profiles. The results showed that *Bifidobacterium, Allobaculum* and *Subdoligranulum* were positively correlated with acetic acid and propionic, while butyric acid was mainly positively correlated with *Bifidobacterium* and *Allobaculum* (Fig. 4e). On the other hand, HDCA was positively correlated with *Bifidobacterium and Ruminococcaceae_Clostridium. Ruminococcaceae_Clostridium* and *Clostridiaceae_Clostridium* presented significant negative associations with 12OH BAs such as TCA and TDCA. Besides, *Allobaculum*, *Subdoligranulum* and *Ruminococcaceae_Clostridium* showed a positive correlation with non-12OH BAs ratio (Fig. 4f). These findings provide evidence for the key role of gut microbiota and its metabolites in whole grain oat-mediated lipid improvement.

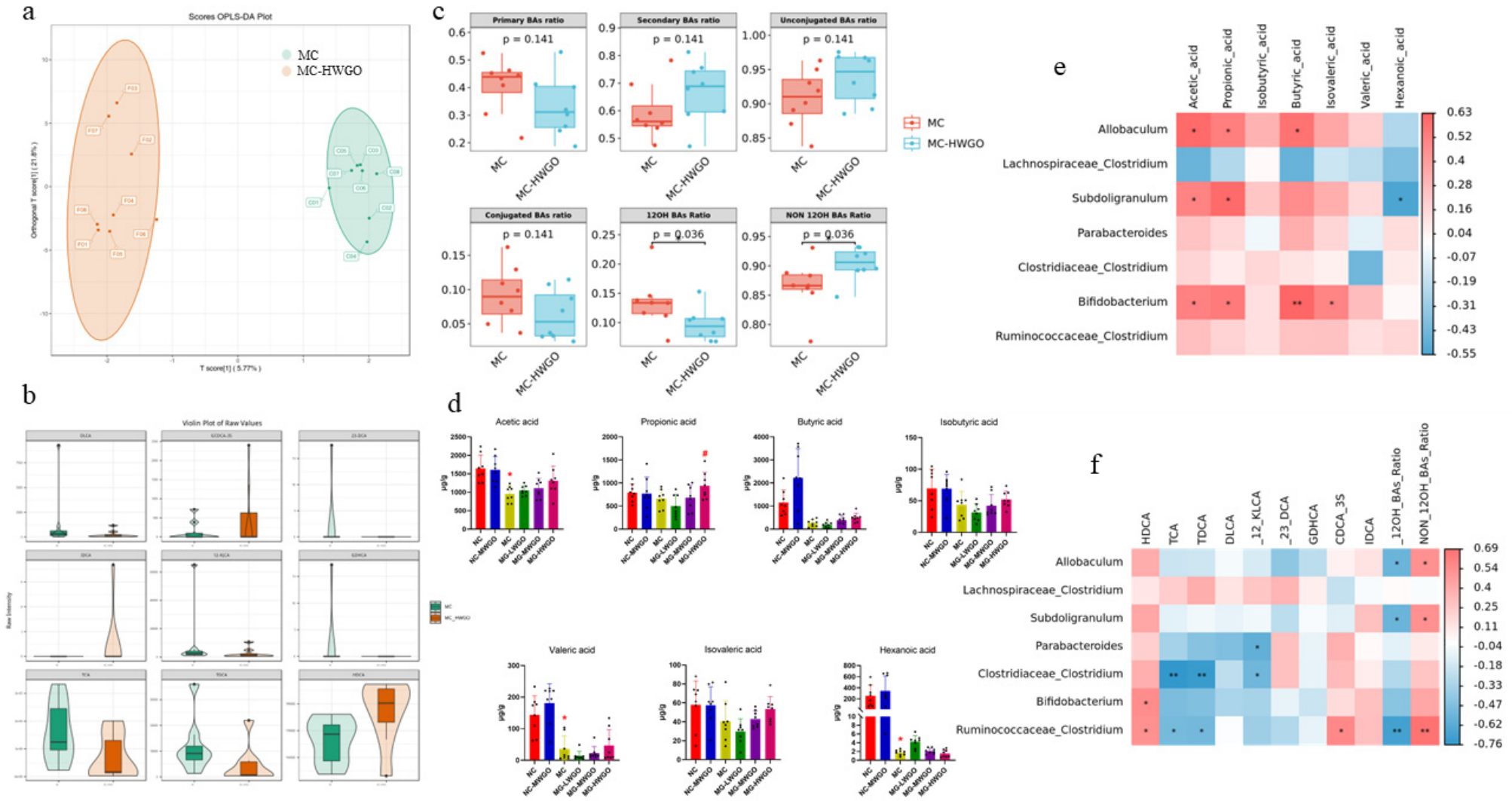

Fig 4. Whole grain oat altered the gut profile of BAs and SCFAs in HFHC-fed rats . **a** The scores of OPLS-DA plot analysis between MC and MC-HWGO group; **b** The modulated cecum BAs classes between MC and MC-HWGO group; **c** Levels of BAs at different classification (main non-12OH BAs include α-MCA, β-MCA, CDCA, LCA, HCA, UDCA, HDCA; others belong to 12OH BAs); **d** The effect of whole grain oat on intestine SCFAs level; **e** Correlations between the significant microbial taxa and intestine SCFAs; f Correlations between the significant microbial taxa and cecum BAs. $^{*}P < 0.05$ compared with NC group, $^{\#}P < 0.05$ compared with MC group, n=8 individuals/group.

2.5 Oral administration non-12OH BAs modulated the protein expression and biochemical parameters in the model rat

Moreover, non-12OH BAs supplementation experiment showed that HDCA treatment inhibited the protein expression level of intestine FXR (Fig. 5a). In addition, the supplementation also induced a significant decrease of serum TC and LDL-C level relative to the HFHC group; supplementation with CDCA and LCA exhibits a decreasing trend on serum cholesterol (Fig. 5b). All these results demonstrated that the effect of whole grain oat on lipid improvement is gut microbiota-dependent, but regardless of the dosage of fecal bacteria donated.

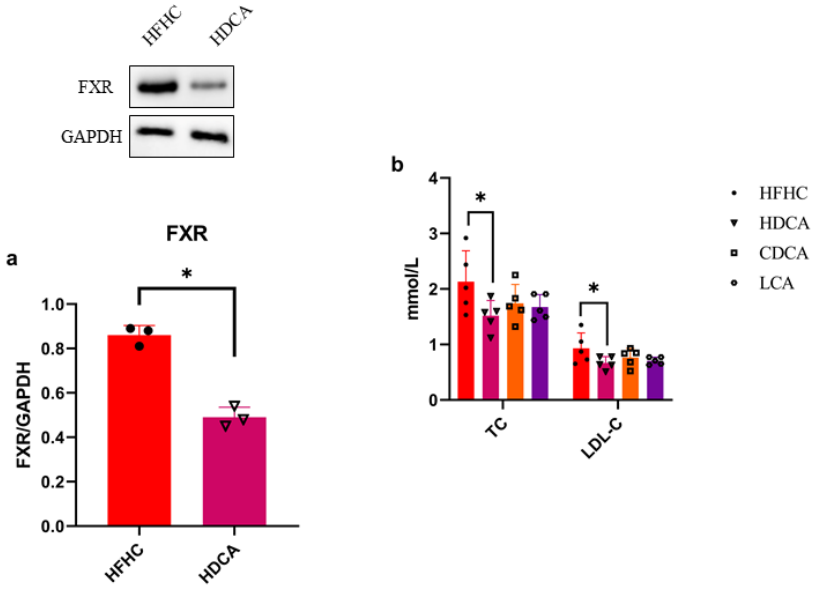

Fig 5. The effect of non-12OH BAs supplementation on intestinal FXR (a) and serum lipid (b). *$P < 0.05$ compared with control group, n=5 individuals/group.

2.6 Cholesterol metabolism-related signaling pathways were activated by whole grain oat

We further detected the protein expression level of signaling pathways related to cholesterol metabolism. The results showed that relative protein levels of LXRα in intestine and CPY7A1 in liver were increased in MC-HWGO group relative to their control, while CYP8B1 protein was not affected (Fig. 6a). Similarly, compared with MC group, MC-HWGO group led to a significant reduction in intestine FXR but an increase in liver CYP27A1; besides, a significantly elevated protein level of CYP8B1 (a limited enzyme determining the ratio of non-12OH BAs) had been found in MC-M/HWGO group (Fig. 6b). All above, we speculated that the classic and alternative pathway were both activated by whole grain oat supplementation, and it seems that non-12OH BAs produced from alternative pathway was dominant on the cholesterol-lowering effect of whole grain oat.

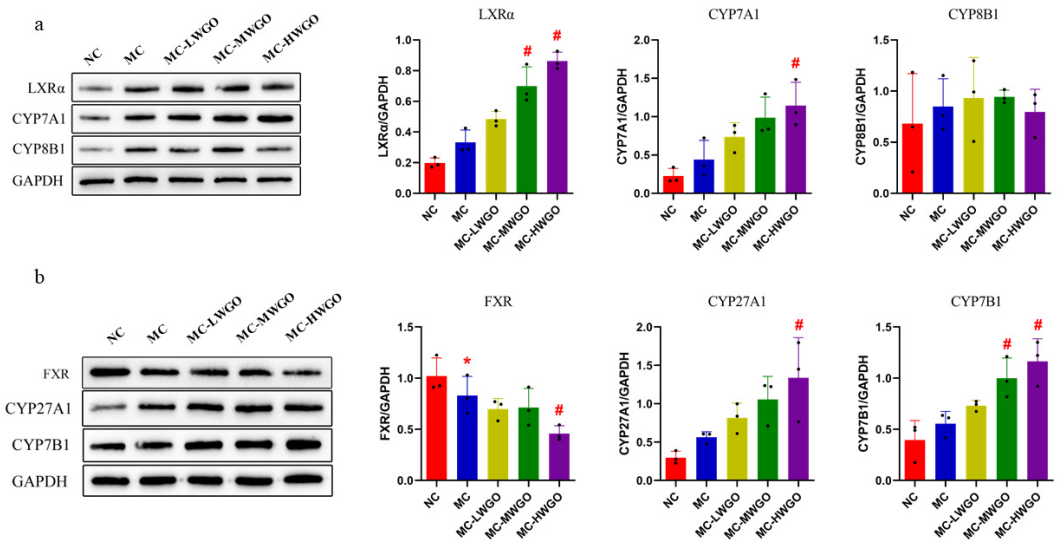

Fig 6. Whole grain oat promoted the expression level of proteins involved in BAs production. a/b Expression levels of proteins involved in BAs synthesis via classic/alternative pathway; $^{*}P < 0.05$ compared with NC group, $^{\#}P < 0.05$ compared with MC group, n=3 individuals/group.

3. Discussion

Besides host genetics, the role of gut microbiota in lifestyle diseases has gained increasing attention. A two-sample mendelian randomization study has indicated a potential causal association between 22 gut microbiota genera and dyslipidemia in humans [37]. In this study, we established a dyslipidemia model by feeding HFHC diets to evaluate the protective effects of whole grain oat supplementation on serum lipid profile and to explore the possible mechanisms. We found that whole grain oat exerted a beneficial effect in modulating serum TC and LDL-C level in a dose-response manner. Also, we demonstrated that gut microbiota induced by whole grain oat play a key role in the attenuation of HFHC-induced experimental dyslipidemia. On the one hand, whole grain oat modulates the structure of gut microbiota, triggering the production of SCFAs, which subsequently inhibit the endogenous synthesis of hepatic cholesterol. On the other hand, the modulated gut microbiota may promote expression of alternative pathway of BA metabolism, this alteration subsequently redirects the transformation of bile acid profiles towards non-12OH BAs, leading to a decreased ratio of 12OH to non- non-12OH BAs, thereby accelerating cholesterol catabolism and exerting a lipid-lowering effect.

Previous studies have demonstrated a direct causal relationship between SCFAs and the improvement of blood lipid levels. [38, 39]. In present study, the MC-HWGO significantly increased the level of propionic acid in feces. We speculated that SCFAs may suppress the activity of hydroxy methylglutaryl coenzyme A (HMG-CoA), which acts as a rate-limiting enzyme during endogenous cholesterol synthesis [30, 40].

BAs constitute a unique class of signaling molecules that interact with the gut microbiota, eliciting a spectrum of physiological responses. Although the gut microbiota can metabolically reshape BA profiles, this process is not direct but mediated by FXR-dependent signaling cascades. For instance, a study demonstrated that, compared with wild-type mice with the same high-fat diet, FXR-defective mice exhibited a significant reduction in *Bacteroidetes* and an increase in *Firmicutes*. Notably, despite these microbial alterations, the BA

profiles remained comparable between groups, suggesting that FXR signaling is essential for microbiota-mediated BA modulation. [41]. Furthermore, FXR, a pivotal BA receptor, plays a vital role in bile acid-mediated regulation of glucolipid metabolism [42]. Specifically, in contrast to 12OH bile acids, non-12OH bile acids can function as FXR inhibitors to promote glucagon-like peptide-1 secretion from enteroendocrine cells, thereby regulating systemic glucolipid metabolism [43]. In our experiments, we explored the difference in the proportions of 12OH BAs and non-12OH BAs among the groups. The results showed that the MC-HWGO group significantly reduced the proportion of 12OH BAs and increased the proportion of non-12OH BAs. Existing studies have demonstrated that a high ratio of non-12OH BAs to 12OH BAs in the BA profile may be beneficial to the high-fat diet induced obesity [44]. CYP8B1 is the only enzyme to produce 12OH BAs, plays a crucial role in determining the ratio between 12OH BAs and non-12OH BAs [45]. For instance, CYP8B1-deficient mice can improve glucose tolerance by increasing the expression of GLP-1, while high levels of 12OH bile acids may promote disorders in glucose metabolism [46, 47]. Furthermore, an increased ratio of non-12OH to 12OH BAs due to the downregulation of CYP8B1 may play a significant role in lipid improvement [48]. In fact, the ratio of non-12OH to 12OH bile acids in the bile acid pool can be modulated through interactions between gut microbiota and hepatic metabolism [49]. It is reported that some bacteria, such as those in the *Clostridiaceae* family, express 7α-hydroxysteroid dehydrogenase and catalyze the 7α→7β epimerization of bile acids, converting chenodeoxycholic acid (CDCA) into ursodeoxycholic acid (UDCA) within the intestinal lumen. For these microorganisms, bile acids with two hydroxyl groups (e.g., CDCA and UDCA) exhibit higher affinity for microbial biotransformation compared to trihydroxy bile acids (e.g., CA). Consequently, these bacteria preferentially metabolize CDCA into UDCA, and the accumulated UDCA further inhibits CYP8B1 activity, which catalyzes 12α-hydroxylation of bile acids. This inhibition reduces the synthesis of 12OH bile acids (e.g., CA), thereby increasing the relative proportion of non-12OH bile acids in the pool [50]. In present study, compared to the NC group, the BAs that decreased in the MC group were all LCA derivatives, which belongs to non-12OH BAs. After whole grain oat intervention, 12OH bile acids such as TCA, TDCA, DCA, and their derivatives decreased significantly. In addition, results from Spearman's correlation analysis showed that the two *Clostridium* families (*Ruminococcaceae_Clostridium* and *Clostridiaceae_Clostridium*) exhibit a significant negative correlation with 12OH BAs such as TCA and TDCA, while displaying a positive correlation with non-12OH BAs like HDCA and CDCA-3S. Meanwhile, *Allobaculum* and *Subdoligranulum*, which is considered to exert beneficial effects in human metabolic diseases [51, 52], also displayed a positive/negative correlation with non-12OH/12OH BAs. Collectively, these findings suggest that the ratio of 12OH to non-12OH BAs can serve as a phenotypic indicator to reflect the degree of lipid disorder.

On the other hand, previous research has indicated that the cholesterol-lowering effects of dietary fiber may be related to the upregulation of intestinal FXR expression, which is inconsistent with the significant inhibition of intestinal FXR by MC-HWGO group. [53, 54]. Given that some studies have indicated a positive correlation between the improvement of hyperlipidemia and the inhibition of intestinal FXR [33, 55], we

speculate that the reduced FXR agonists, like DCA, observed in our study, may suppress intestinal FXR expression; meanwhile the enhanced non-12OH bile acids, such as HDCA, CDCA-3S, may activate liver LXR expression, ultimately enhancing BAs synthesis and metabolism in the liver.

Of note, we found that the expression of CYP7A1 and CYP27A1 in MC group presented a non-significant increase in comparison with NC group. A possible explanation is that, when facing adverse environment such as high-fat load, body can promote BAs metabolism to against this downside, which may represent a compensatory response of the body itself [56]. Our hypothesis was further supported by the study conducted by Hu et al., in which the authors found that a high-fat load diet did increase the mRNA level of CYP7A1; furthermore, a higher expression of CYP7A1 was observed after corn bran dietary fiber intervention [53]. However, it should be acknowledged that whether the limited compensatory response found in present study would affect the regulatory effect of whole grain oat on blood lipid is unclear, and further study is still required.

FMT can provide novel intuitive evidence for the mechanisms of gut microbiota-related diseases [57]. Thus, we conducted validation experiments to verify whether the impacted gut microbiota and its metabolites was the results or the reason of alleviated dyslipidemia. After FMT, we found that the microbial community in the recipient rats exhibited similar to their donors, and significant improvements in serum TC and LDL-C were also observed although the remission is independent on the dosage of transplanted fecal suspension. Besides, we have demonstrated that non-12OH BAs such as HDCA administration alleviates dyslipidemia in a rodent model induced by HFHC diet. In fact, HDCA has been proven to enhance the capacity of body against a wide range of metabolic diseases [58, 59]. Collectively, our results have indicated that the modulated gut microbiota and the alterations in its metabolites are essential in whole-grain oat-mediated improvement of blood lipids.

There are some limitations that should be taken into consideration. First of all, despite that we have identified the crucial role of *Clostridium* family in the production of non-12OH BAs, if supplementation with *Clostridium* family or FMT is effective for enhancing the production of non-12OH BAs remains to be investigated in future study. Another limitation refers to the choice of BAs for oral administration study. Although HDCA was selected in this study and subsequently confirmed to attenuate dyslipidemia upon oral administration into HFHC-induced dyslipidemia, other significant abundant non-12OH BAs are likely to play a role as well. It will be important to evaluate the efficacy of other non-12OH BAs in mitigating diet-related dysmetabolism.

4. Conclusion

In summary, we demonstrated that whole grain oat can protect against HFHC-induced dyslipidemia via gut-liver axis. Further mechanistic study suggests that whole grain oat reverses the gut microbiota dysbiosis and promotes the production of propionate. Besides, the modulated gut microbiota can activate the alternative pathway of BAs metabolism and increase the proportions of non-12OH in total BAs pool. The FMT

experiment unveils the causal relationship between the cholesterol-lowering properties of whole grain oat and the gut microbiota. The proposed mechanisms are summarized in Fig. 7.

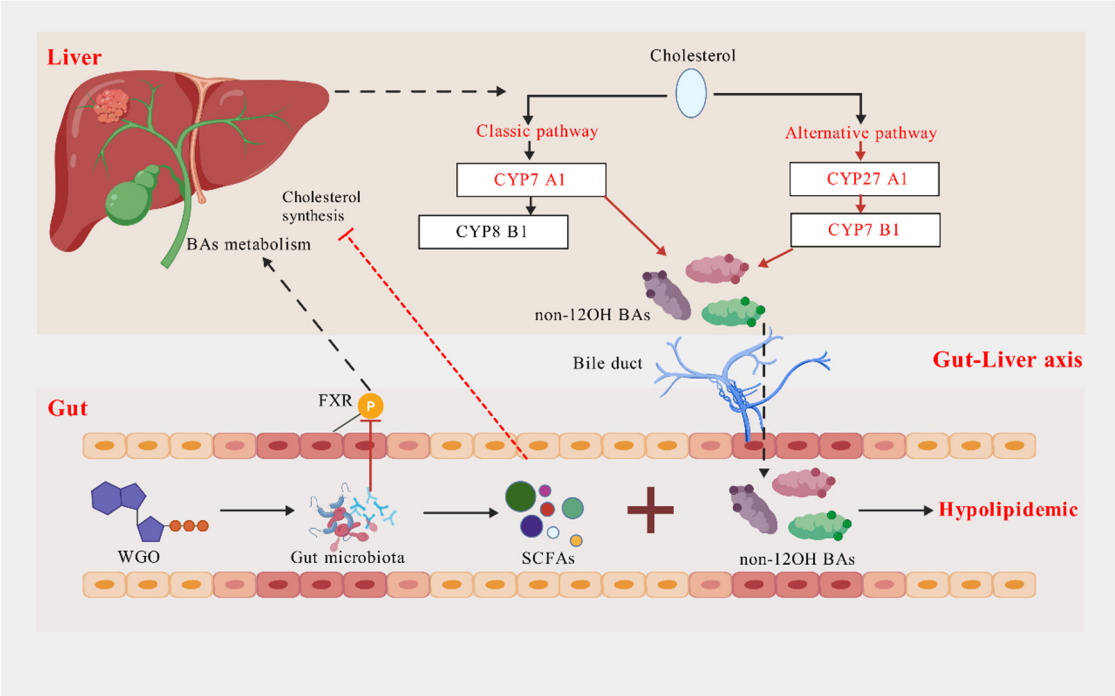

Fig 7. The proposed mechanism for whole grain oat reduces cholesterol level.

5. Method

5.1 Chemicals and reagents

HFHC diet (Research diet: D12109C) contained 40% energy from fats, 40% energy from carbohydrates, 20% energy from proteins (partially substituted with whole grain oat with 5g/100g, 10g/100g and 20g/100g), while the control diet (Research diet: D12102C) contained 10% energy from fats, 70% energy from carbohydrates, 20% energy from proteins. Whole grain oats were supported by a Quaker Oats manufacturing facility (Shanghai, PepsiCo, China). The antibodies used for western blot were: LXRα (ProteinTech, 4351-1-AP), CYP7A1 (Affinity, DF2612), CYP8B1 (Abcam, ab191910), FXR (ProteinTech, 13194-1-AP), CYP27A1 (ProteinTech, 14739-1-AP), CYP7B1 (Abcam, ab138497), GAPDH (Abcam, ab8245), HRP anti-mouse antibody (Abcam, ab6789), HRP anti-rabbit antibody (Abcam, ab6271). BAs and SCFAs standards were obtained from CNW Technologies GmbH and Sigma respectively. Antibiotics were purchased from Macklin.

5.2 Animal and study design

All the procedures in present study were conducted under the national legislation and approved by the Laboratory Animal Committee of Southeast University (NO. 20230318002). 6-week-old SPF Sprague Dawley (SD) rats were purchased from Beijing Charles River laboratory Animal Technology Co., Ltd. All the rats were maintained in a controlled environment with food and water ad libitum under a 12h light/dark cycle at 20℃ -22℃ and 40% ± 5% humidity.

In the whole grain oat intervention experiment, 6-week-old SD rats were acclimated by feeding a control chow diet for 1 week, and then were randomly assigned into 6 groups according to the body weight, 8 rats in each group: normal control (NC) that received control chow (D12102C), 10% whole grain oat intervention (NC-MWGO) that received 10% whole grain oat-substituted control chow, model control (MC) that received HFHC diet (D12109C), 5%/10%/20% whole grain oat intervention (MC-L/M/HWGO) that received 5%/10%/20% whole grain oat-substituted HFHC diet, respectively. Body weights and food intakes were recorded regularly during 12-week intervention.

In the FMT study, in order to establish a germ-free rat model, rats in recipient group received 2 weeks of antibiotic treatment (metronidazole 1 g/L + vancomycin 0.5 g/L + neomycin sulfate 1 g/L + ampicillin 1 g/L dissolved in drinking water combined with gavage daily) after 12-week HFHC feeding, and then were divided into 4 groups (4 each group), housed in sterile plastic package isolators, and were oral administration of 400μL or 800μL of fecal suspension. The gavage was performed once every two days to reinforce the microbiota transplantation for 1 week. The microbiota donor were the rats treated with MC or MC+ HWGO for 12 weeks. Fecal of the donors were collected and dispersed in sterile Ringer working buffer, then centrifuging (2500 r/min) for 1min and take the supernatants for transplantation. The transplanted rats were then supplied with HFHC diet for another 3 weeks (The study design was shown in Supplementary Fig.1). Blood samples and fecal samples were collected at the end of the experiment for analysis of circulating lipids and BAs respectively.

In the non-12OH BAs supplementation experiment, 6-week-old SD rats were randomly divided into 4 groups, 5 rats in each group: (1) vehicle fed HFHC diet (HFHC group), (2) HFHC coupled with 100mg/kg body weight of HDCA by gavage (HFHC+HDCA group), (3) HFHC coupled with 100mg/kg body weight of CDCA by gavage (HFHC+CDCA group), (4) HFHC coupled with 50mg/kg body weight of LCA by gavage (HFHC+LCA group). The interventions were performed for 8 weeks.

5.3 Measurement of serum lipids parameters

Briefly, Serum TG and TC were measured by Mindray BS-800 automatic blood biochemical analyzer. HDL-C and LDL-C were detected by enzymatic methods using commercial assay kits (Institute of Biological Engineering of Nanjing Jiancheng, China), according to manufacturer's protocol.

5.4 Fecal samples collections and 16S rRNA sequencing

Fecal specimens were collected after sacrificing in a sterile environment, all the samples were snap-frozen in liquid nitrogen before storage at -80°C. Total genomic DNA samples were extracted by using the FastDNA SPIN Kit (MP Biomedicals, USA) according to the provided protocol. Agarose gel electrophoresis and Nanodrop were employed to measure the purity and integrity of the DNA.

The V3-V4 regions were amplified with the 16S rDNA gene universal primers. Sequencing was performed on Illumina Hiseq 2500 platform. The Quantitative Insights into Microbial Ecology 2 (QIIME2) pipeline was used to process the sequencing data. Briefly, we used DADA2 to achieve the quality control and denoising with the default parameters, then we clustered the remaining high-quality sequences into

operational taxonomic units (OTUs) at 97% sequence similarity (based on the Greengenes reference database). α-diversity were calculated by QIIME2. Fast UniFrac PCoA was conducted with the phylogenetic tree constructed by inserting the representative of each OUT.

5.5 Detection of fecal SCFAs profiles

Take sample into the 2 mL EP tubes, extracted with 1 mL H_2O, vortex mixing for 10 s. Homogenized in ball mill for 4 min at 40 Hz, then ultrasound treated for 5 min (incubated in ice water), repeat 3 times. Centrifuge for 20 min at 5000 r/min, 4℃. Transfer the supernatant 0.8 mL into a fresh 2 mL EP tubes; Add 0.1 mL 50% H_2SO_4 and 0.8 mL of extracting solution (25 mg/L stock in methyl tert-butyl ether) as internal standard, vortex mixing for 10 s, oscillations in 10 min, then ultrasound treated for 10 min (incubated in ice water). Centrifuge for 15 min at 10000 r/min, 4℃. Keep at -20 ℃ for 30 min. Transfer the supernatant into a fresh 2 mL glass vial, for GC-MS analysis.

SHIMADZU GC2030-QP2020 NX gas chromatography-mass spectrometer was used to detect the SCFAs. The system utilized a HP-FFAP capillary column (30m × 250μm × 0.25μm, J&W Scientific, Folsom, CA, USA). A 1μL aliquot of the analyte was injected in split mode (5:1). Helium was used as the carrier gas, the front inlet purge flow was 3 mL min^{-1}, and the gas flow rate through the column was 1 mL min^{-1}. The initial temperature was kept at 50 ℃ for 1 min, then raised to 150 ℃ at a rate of 50 ℃ min^{-1} for 1 min, then raised to 170 ℃ at a rate of 10 ℃ min^{-1} for 0 min, then raised to 210 ℃ at a rate of 20 ℃ min^{-1} for 1 min, then raised to 240 ℃ at a rate of 40 ℃ $min^{-1`}$ for 1 min. The injection, transfer line, quad and ion source temperatures were 220 ℃, 240 ℃, 150 ℃ and 200 ℃. The energy was -70 eV in electron impact mode. The mass spectrometry data were acquired in Scan/SIM mode with the m/z range of 33-150 after a solvent delay of 3 min.

5.6 Quantitative analysis of BAs

5.6.1 Sample preparation and extraction

Samples (cecum contents, 20mg) were extracted with 200μL methanol/acetonitrile(v/v=2:8) after the samples grinded with ball mill. 10μL internal standard mixed solution (1μg/mL) was added into the extract as internal standards (IS) for the quantization. Put the samples at -20℃ for 10 min to precipitated protein. Then centrifugation for 10min (12000r/min, and 4℃), the supernatant was transferred to clean plastic microtubes. The extracts were evaporated to dryness, reconstituted in 100μL 50% methanol (V/V) for further LC-MS analysis.

5.6.2 HPLC Conditions

The sample extracts were analyzed using an LC-ESI-MS/MS system. The analytical conditions were as follows, HPLC: column, Waters ACQUITY UPLC HSS T3 C18 (100mm × 2.1mm i.d., 1.8μm); solvent system, water with 0.01% acetic acid and 5mmol/L ammonium acetate (A), acetonitrile with 0.01% acetic acid (B); The gradient was optimized at 5% to 40%B in 0.5min, then increased to 50%B in 4 min, then increased to 75% B in 3 min, and then 75% to 95% in 2.5 min, washed with 95%B for 2 min, finally ramped back to 5%B

(12-14 min); flow rate, 0.35 mL/min; temperature, 40℃; injection volume: 1μL. The effluent was alternatively connected to an ESI-triple quadrupole-linear ion trap (QTRAP)-MS.

5.6.3 ESI-MS/MS Conditions

Linear ion trap (LIT) and triple quadrupole (QQQ) scans were acquired on a triple quadrupole-linear ion trap mass spectrometer (QTRAP), QTRAP® 6500+ LC-MS/MS System, equipped with an ESI Turbo Ion-Spray interface, operating in negative ion mode and controlled by Analyst 1.6.3 software (Sciex). The ESI source operation parameters were as follows: ion source, ESI-; source temperature 550 ∘C; ion spray voltage (IS) -4500 V; curtain gas (CUR) was set at 35 psi, respectively. Bile acids were analyzed using scheduled multiple reaction monitoring (MRM). Data acquisitions were performed using Analyst 1.6.3 software (Sciex). Multiquant 3.0.3 software (Sciex) was used to quantify all metabolites. Mass spectrometer parameters including the declustering potentials (DP) and collision energies (CE) for individual MRM transitions were done with further DP and CE optimization (**Supplementary Table 1**). A specific set of MRM transitions were monitored for each period according to the metabolites eluted within this period.

5.7 Western blotting analysis

Intestine and liver tissues (30~50mg) from experimental rats were homogenized with RIPA buffer (Beyotime Technology, Shanghai, China) containing 1% PMSF in an ice bath, then followed by centrifugation at 12,000 g for 5 min to harvest the cleared lysates. Protein quantification was measured by using a bicinchoninic acid (BCA) protein assay kit (Beyotime Technology, Shanghai, China) with bovine serum albumin as a standard. An equal amount of protein sample was separated by 10% SDS–PAGE protein loading buffer, and then transferred to a polyvinylidene fluoride membranes (Millipore, Billerica, MA, USA). Subsequently, the membrane was blocked with skimmed milk at room temperature for 1 h, probed overnight at 4 ℃ with primary antibodies and then incubated with HRP-conjugated secondary antibodies. The blots were visualized using a Tanon 5,500 Chemiluminescent Imaging System (Tanon Science & Technology Co., Shanghai, China). The gray values of the bands were calculated by using ImageJ software (Version 1.5, USA) and were normalized to β-Actin, and were furtherly presented as fold changes relative to the control value.

5.8 Statistical analysis

Results were displayed as mean ± SD if it follows a normal distribution. Otherwise, median and interquartile range (IQR) was used. All data were analyzed by using the one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison's test. Spearman's correlation analysis was employed to examine relationships between key gut microbiota and BAs, SCFAs in both groups. All analyses were performed by using SPSS version 18.0 (SPSS Inc, USA) with a 5% level of significance.

Supplementary files

Supplementary Fig 1. The study design of FMT experiment

Supplementary Fig 2. The quality control of 16S rRNA sequencing

Supplementary Fig 3. The effect of antibiotics treatment on gut microbiota

Supplementary Fig 4. The effect of FMT on gut microbiota

Supplementary Fig 5. The significant differences in bile acid profiles between NC and MC group

Supplementary Fig 6. The cecum BAs classes level of rats between NC and MC group

Supplementary Table 1. Optimized MRM parameters for the quantification of key bile acid metabolites

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Availability of data and materials

The raw data of 16S rRNA were deposited into the NCBI Sequence Read Archive (SRA) database (Accession number: PRJNA1062159). Other data generated or analyzed during this study are included in this published article [and its supplementary information files].

Ethics approval and consent to participate

All the procedures in present study were conducted under the national legislation and approved by the Laboratory Animal Committee of Southeast University (NO. 20230318002).

Competing interests

The authors declare that they have no competing interests.

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