

Pueraria lobata resistant starch improves obesity by regulating lipid metabolism and gut microbiota in high-fat diet mice

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Abstract

The health potential of resistant starch (RS) on the gut and its microbiota has received widespread attention. Here, we prepared *Pueraria lobata* resistant starch (PLRS) by modifying *Pueraria lobata* starch with *Pullulanase* and hypothesized that PLRS could improve lipid metabolism disorders and modulate gut microbiota structure in high-fat diet (HFD) induced obese mice. Our results revealed that PLRS alleviated lipid metabolism disorders by ameliorating HFD-induced liver and intestinal damage, significantly upregulating the abundance of *Akkermansia*, *Lactobacillus*, *Blautia*, and *Dubosiella*, as well as markedly suppressing *Staphylococcus* in obese mice. Molecular mechanisms have elucidated that the PLRS may exert reducing fat accumulation and stabilize the gut microbiota effects through the modulation of the fatty acid metabolic signaling pathway and the PPAR signaling pathway. Therefore, we suggest that this prebiotic carbohydrate with specific physiological functions could be used to prepare healthy lipid-lowering foods to attenuate obesity problems.

Introduction

Obesity, as a disease with the number of sufferers increasing every year, has become a serious public health problem in the world, in which fat accumulates continuously, causing triglycerides, cholesterol, and other biochemical indicators to deviate from normal levels caused by the breakdown of the balance between energy intake and output [1]. It has been found that obesity was mainly caused by genes, poor lifestyles, and diet habits, and was related to lipid metabolism disorders, intestinal flora imbalance, and inflammation [2]. The persistence attached to elevated obesity would increase the risk of Alzheimer's disease, cancer, cardiovascular and cerebrovascular diseases, non-alcoholic fatty liver, and more [3]. However, weight loss increases a variety of uncomfortable reactions, which further increase energy intake and decrease energy expenditure [4]. In recent studies, gut microbiota has been found to be closely associated with these multitudes of chronic metabolic diseases, and the search for specific probiotics is becoming a major research priority [5]. For this reason, resistant starch, which cannot be digested and absorbed, is a promising strategy.

Pueraria lobata (PL), as a traditional Chinese medicinal material for both food and medicine, has extremely high nutritional value [6]. PL was not only rich in various mineral elements such as phosphorus, calcium, potassium and iron, but also its starch content was higher than 20% [7]. RS has a wide commercial value due to the special physical and chemical properties. Generally, the use of RS as a food ingredient does not alter the taste of the food, and it exhibit better organoleptic characteristics in some products due to the swelling, water absorption, and freeze-thaw stability [8]. RS has shown the strong medical value. RS modified in different ways could not be digested and absorbed by the small intestine [9]. But it could be fermented by gut microbiota to produce short-chain fatty acids, amino acids, ceramide, and so on, which had physiological activities such as regulating blood sugar, reducing fat, anti-inflammatory, preventing colon cancer, enhancing immunity and more [10]. Thus, focusing on the wider evidence of the health effects from RS will help generate significant economic and social benefits.

Therefore, this study aimed to evaluate the effect of *Pueraria lobata* resistant starch (PLRS) on physiological indicators, gut microbiota composition and molecular mechanisms in HFD-fed mice. Meanwhile, the relationship among mice model, gut microbiota, and lipid metabolism was analyzed to explore the mechanism of lipid metabolism.

Materials And Methods

The material and methods section were presented as supplementary information.

Results

Effect of PLRS on biochemical indicators

Effects of PLRS on body weight, visceral fat accumulation, blood glucose and lipid levels, and histopathology in mice were presented as supplementary information.

Effect of PLRS on hepatic impairment

ALT and AST were the two main enzymes in the human liver, which used to evaluate the liver function. TBA is a class of water-soluble compound produced by the catabolism of cholesterol in liver. To explore the effect of PLRS on the lipid metabolism, the levels of ALT, AST, and TBA were measured in HFD-induced mice. As shown in Fig.1. HFD mice had high level of ALT, AST, and TBA compared with the NFD group ($P \leq 0.01$). Instead, the levels of various indicators decreased after treatment with Orlistat and PLRS, and the down-regulation effect of ALT and AST in the HFD+HP group is better than that of the HFD+Orli group. The above description showed that PLRS could improve HFD cause liver damage by down-regulating the levels of ALT, AST, and TBA.

PLRS determination of intestinal contents by 16S rDNA

The changes of gut microbiota structural was studied by high-throughput Illumina HiSeq sequencing of the V3-V4 region of the 16S rRNA gene. The PCA score plot showed that the microbiota composition of cecum content samples differed significantly between the NFD and HFD groups (Fig.2a). The results of the α -diversity analysis suggested that both HFD+LP and HFD+HP groups significantly increased the diversity index of Simpson and Chao1, however, the HFD+Orli decreased the richness of the Chao1 index (Fig.2b-c). The results suggested that PLRS enhances the diversity on HFD mice. At the phylum level, a total of 10 phyla were identified.

Firmicutes, *Bacteroidetes*, *Proteobacteria*, *Verrucomicrobia*, *unidentified_Bacteria*, *Actinobacteria*, *Chloroflexi*, *Deferribacteres*, *Acidobacteria*, and *Cyanobacteria* the major gut microbiota in all groups. In the NFD group, the main gut microbiota was dominated by *Bacteroidetes* (40.56%), *Firmicutes* (37.99%), and *Proteobacteria* (17.11%), however, the proportion in the HFD group changed to *Firmicutes* (56.18%), *Bacteroidetes* (33.35%), and *Proteobacteria* (22.07%), respectively (Fig.2d). After the Orlistat and PLRS treatment, the number of *Firmicutes* decreased compared with the HFD group. More importantly, the

Firmicutes to *Bacteroidetes* (F/B) ratio as the two main bacterial phyla, which is widely used as a biomarker for predicting obesity (Fig.2e). Compared with the HFD group, the ratio of HFD+Orli, HFD+LP, and HFD+HP were reduced but without statistically significant. In the second stage, the abundance of gut bacteria was analyzed and compared separately at the genus level (Fig.2f). Compared with the NFD group, The HFD reduced the abundance of *Ruminiclostridium*, *Helicobacter*, and *Alloprevotella* in the intestine, while in the HFD group, the content of *Staphylococcus* was significantly increased relative to the other groups. In PLRS groups, we observed a significant increase in the relative abundance of *Akkermansia*, *Lactobacillus*, *Blautia*, and *Dubosiella* and a significant decrease in the abundance of *Staphylococcus*. Interestingly, at the genus level, Orlistat did not increase the abundance of intestinal flora but only decreased the abundance of *Alloprevotella* and *Staphylococcus*.

PLRS consumption regulated hepatic lipid metabolism genes and proteins.

HFD leads to lipid deposition in serum and liver, possibly due to differences in the expression levels of genes and proteins involved in the regulation of hepatic lipid metabolism. Based on this, we investigated mRNA and protein expression of 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR), sterol regulatory element-binding protein-1c (SREBP-1c), cholesterol 7 α -hydroxylase (CYP7A1), and fatty acid synthase (FAS) in the fatty acid metabolic pathway and the PPAR metabolic pathway.

As shown in Fig.3a, the mRNA expression levels of HMGCR, SREBP-1c, and FAS were increased in HFD group as compared with the NFD group, and the increased level of SREBP-1c and FAS were significant ($P \leq 0.01$). After Orlistat and PLRS gavage treatment, the mRNA expression was down-regulated. The mRNA expression level of CYP7A1 was down-regulated significantly in HFD mice compared with the NFD group ($P \leq 0.05$). Nevertheless, PLRS supplementation significantly increased the levels of these genes compared with the HFD group. The up-regulation level of the CYP7A1 gene was higher in the HFD+HP group compared with the HFD+Orli group.

As shown in Fig.3b-c, compared with the NFD group, HFD mice showed significantly increased protein expression levels of HMGCR, SREBP-1c, and FAS ($P < 0.01$), and significantly decreased protein expression of CYP7A1 ($P < 0.01$); Compared with the HFD group, PLRS groups showed significant differences in regulating the expression levels of other proteins, CYP7A1 especially, which was even better than the NFD group, suggesting that PLRS may have the ability to target CYP7A1 to reduce obesity symptoms.

Correlation analysis of gut microbiota and lipid metabolism

To investigate the underlying interaction of PLRS on HFD-induced intestinal flora and lipid metabolism, the pearson algorithm was further used to assess the association among intestinal flora and serum biochemical indicators and key genes and proteins in the liver, as shown in Fig.4. *Staphylococcus* is positively correlated with hepatic TBA, levels of AST in the liver were positively correlated with the abundance of *Bacteroides* and *unidentified_Clostridiales*, while serum levels of TG were negatively correlated with the abundance of both *Staphylococcus* and *Alloprevotella*, the abundance of *Alloprevotella* and *Akkermansia* increased with increasing levels of ALT. In addition, the abundance of *Akkermansia* was highly correlated with both of the gene and protein expression level of SREBP-1c, and that elevated levels of FAS expression were linked with increased abundance of *Alloprevotella*, *Staphylococcus*, and *Dubosiella*. By contrast, CYP7A1 expression was in a negative correlation with the abundance of *Akkermansia*, *unidentified_Lachnospiraceae*, and *Dubosiella*. These results suggest that PLRS can inhibit the disruption of lipid metabolism caused by HFD, possibly due to involvement in pathways related to microbiota that can regulate lipid metabolism and improve intestinal damage by increasing the abundance of these beneficial bacteria in the gut.

Discussion And Conclusion

In this study, the tendency of increased body weight of HFD mice was suppressed by PLRS-treated. At the same time, the weight of the kidney, liver, pWAT, gWAT, and iWAT have been reduced. Interestingly, there was no significant difference in the level of food intake, which means that PLRS does not achieve the purpose of anti-obesity by controlling appetite, and it could be the key step in the process of studying the anti-obesity mechanism of PLRS. HDL-C is involved in the reverse transport of TC, the levels of parameters related to dyslipidemia were significantly reduced by PLRS [11]. The liver maintains a steady-state in the synthesis, intake, excretion, and transportation of TC and TG, which means that PLRS plays an important role in maintaining the stability of lipid metabolism [12]. In addition, it was found that supplementing PLRS to HFD-induced mice could also significantly reduce the levels of ALT, AST, and TBA. The three indicators are relevant evidences of the presence of liver pathology, and obese patients are outside the normal range [13]. Our results show that PLRS intake is effective in reducing body weight, lowering blood and liver lipid levels to ameliorate liver damage.

The capability of PLRS in regulating the gut was further explored by measuring the abundance of intestinal flora. At the phylum level, *Firmicutes*, *Bacteroidetes*, and *Proteobacteria* were the main microorganisms in the intestine. A high ratio of *Firmicutes* to *Bacteroidetes* predisposes to obesity [14]. Chen et al. found significant antibacterial activity of extracts PL, which may be a potential cause of PLRS inhibition of *Staphylococcus* in our study [15]. Thus, we found that PLRS repaired intestinal damage and suggested that PLRS could upregulate *Akkermansia*, *Lactobacillus*, *Blautia*, and *Dubosiella*, and downregulate *Staphylococcus* to restoring HFD-induced intestinal inflammation, reducing the lipid layer of the intestine and improving the ability of the intestine to ferment.

FAS was a fatty acid synthesis gene as a downstream factor of the SREBP-1c gene [16]. At the same time, HMGCR and CYP7A1 are different key rate-limiting enzymes, with HMGCR responsible for the de novo synthesis of cholesterol and CYP7A1 responsible for the further synthesis of cholesterol into bile acids [17]. Bile acids are re-transported back to the liver via the portal vein during enterohepatic circulation [18]. Therefore, our results demonstrated that PLRS first downregulates SREBP-1c and FAS to inhibit TG synthesis and reduce excessive fat accumulation in obese mice through the fatty acid metabolic pathway. Then through the PPAR signaling pathway downregulating HMGCR to inhibit TC synthesis, and increasing CYP7A1 level to ensure adequate bile acids and promotes a homeostatic environment in the enterohepatic circulation by increasing abundance of beneficial microbiota.

Overall, our study mainly confirms the anti-obesity mechanism of PLRS in HFD mice. HFD-induced hazard effects and the health effects of oral PLRS were firstly determined by weight, lipids, liver markers and tissue sections. Next, the alterations in the gut microbiota of obese mice by HFD and PLRS were identified. We found that HFD induced a decrease in the abundance of *Ruminiclostridium*, *Helicobacter*, and *Alloprevotella* and an increase in *Staphylococcus*, while PLRS upregulate *Akkermansia*, *Lactobacillus*, *Blautia*, and *Dubosiella*, and downregulate *Staphylococcus*. Finally, the expression levels of the key factors of the fatty acid metabolic signaling pathway and the PPAR signaling pathway were determined, and the correlation among the gut microbial and biochemical indicators and the key factors. In the fatty acid metabolic pathway, PLRS down-regulates SREBP-1c and FAS to inhibit TG synthesis. In the PPAR pathway, down-regulates HMGCR to inhibit TC synthesis and increases CYP7A1 to regulate bile acids. During these processes, excess TBA levels and *Staphylococcus* were highly positively correlated, pointing to increased inflammation in HFD-induced obese mice as a result of increased *Staphylococcus*. However, intake of PLRS reversed this result.

In short, characteristics of obesity as a disorder over lipid metabolism, closely related to liver damage and gut microbiota. Our study has provided a theoretical basis for the development of innovative food products with the potential to improve lipid metabolism disorders and regulate gut health. Nevertheless, PLRS further should be evaluated for potential value in human experiments.

Declarations

Funding

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Conflict of Interest

The authors declare that they have no conflict of interest.

Data Availability

Not applicable.

Authors' contributions

Na Li and Quan-cen Li conducted the study; Xiao-xiang Gao, Xiao-dong Ge and Zi-feng Huang, drafted the first manuscript; Wen-wen Cai and Fu-jie Chen analyzed the data; Bin Liu and Feng Zeng conceived and designed the study, critically reviewed the manuscript. All authors approved the final manuscript for submission.

Ethics approval

The animal experiment and procedure have been approved by the ethics review committee of the College of Food Science, Fujian Agriculture and Forestry University (FS-2021-025).

Consent to participate

The present paper has been approved by all named authors.

Consent for publication

The present paper, which is original, has not been published before and is not currently being considered for publication elsewhere.

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Figures

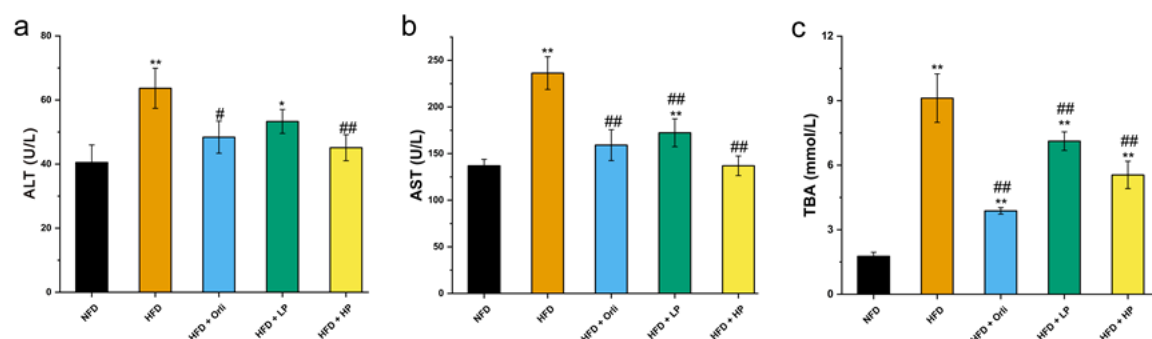


Figure 1

Effects of orally administered Orlistat and PLRS on the levels of (a) ALT, (b) AST, and (c) TBA in HFD mice. Data are expressed as mean ± SD. * $p < 0.05$ and ** $p < 0.01$ compared with the NFD group; # $p < 0.05$ and ## $p < 0.01$ compared with the HFD group.

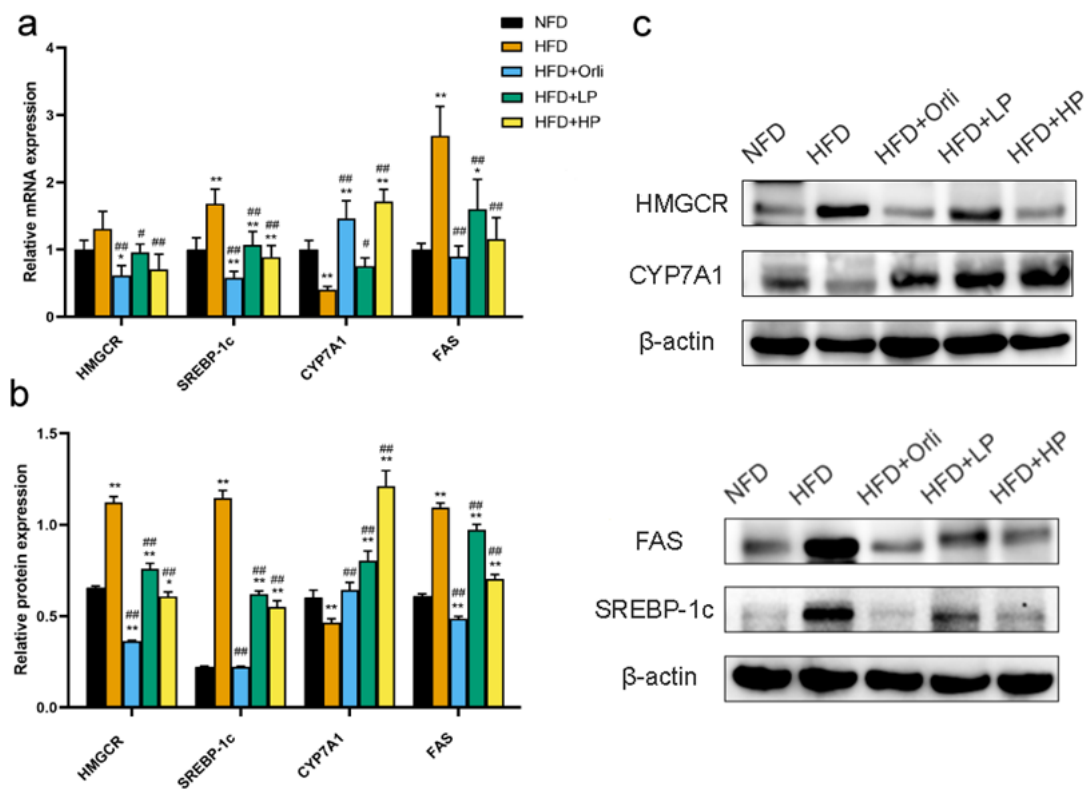


Figure 3

Effects of orally administered Orlistat and PLRS on the expression levels of (a) genes, (b-c) and proteins in HFD mice. Data are expressed as mean $\pm$ SD. * $p < 0.05$ and ** $p < 0.01$ compared with the NFD group; # $p < 0.05$ and ## $p < 0.01$ compared with the HFD group.

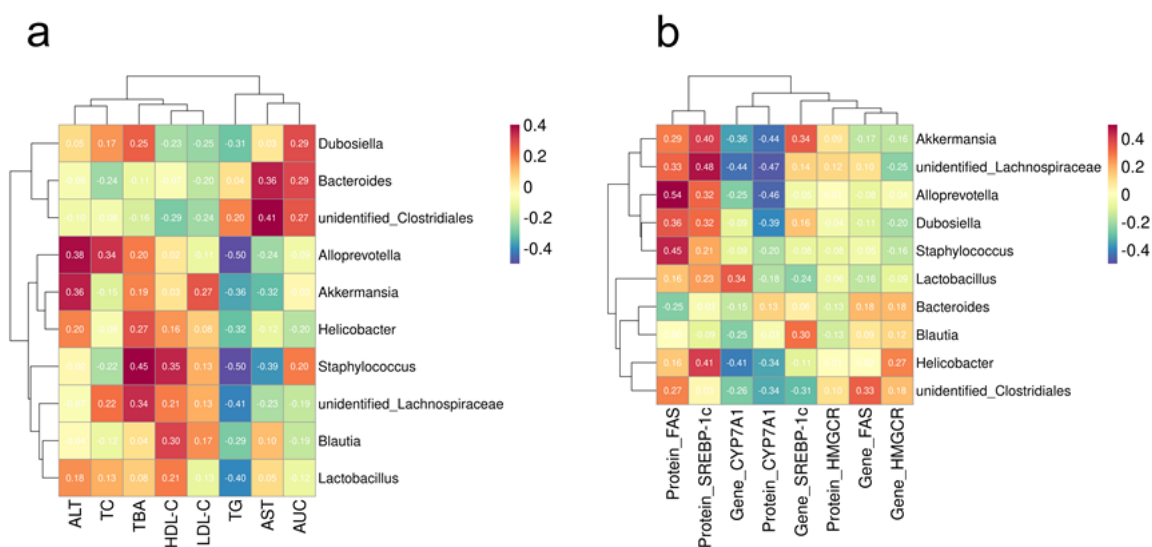


Figure 4

Correlations between gut microbial composition and lipid metabolism parameters. (a) Correlation of biochemical indicators with gut microbial composition, (b) Correlation of genes and protein levels with gut microbial composition.

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