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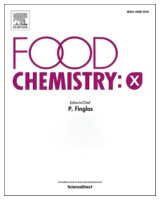

Sulfur dioxide-free wine with polyphenols promotes lipid metabolism via the Nrf2 pathway and gut microbiota modulation

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ABSTRACT

Moderate wine consumption is often associated with preventing obesity, yet concerns arise due to the health risks linked to its constituent antioxidant, SO_2. Recent focus has turned to polyphenols as a potential substitute for SO_2. This investigation explores the impact and mechanisms of sulfur dioxide-free wine enriched with polyphenols on lipid regulation. Through a comprehensive analysis involving oxidative stress, lipid metabolism, and gut microorganisms in high-fat-diet mouse models, this study reveals that sulfur dioxide-free wine containing the polyphenol resveratrol exhibits a heightened ability to regulate lipids. It modulates oxidative stress by influencing NF-E2-related factor 2, a crucial factor, while enhancing lipid metabolism and fatty acid β-oxidation through key genes such as carnitine palmitoyltransferase I and peroxisome proliferator-activated receptor alpha. Furthermore, oral administration of sulfur dioxide-free wine supplemented with resveratrol demonstrates an increase in the relative abundance of beneficial intestinal microflora, such as *Turicibacter*, *Allobaculum*, *Bacteroides*, and *Macellibacteroides*, while decreasing the Firmicutes/Bacteroidetes ratio.

1. Introduction

Obesity represents a complex and chronic disturbance in metabolic processes primarily driven by an energy imbalance. It is intricately associated with various chronic conditions, such as cancer, hyperlipidemia, type 2 diabetes, inflammation, and cardiovascular issues (Gasmi et al., 2021; Kolb et al., 2016; Yeh et al., 2021). Establishing a routine of consuming functional meals plays a pivotal role in preventing obesity. Studies consistently highlight the significant potential of polyphenol-rich foods in developing effective strategies to manage obesity (Dzah et al., 2023).

Wine, rich in bioactive polyphenols, has been extensively studied for its positive effects on regulating obesity when consumed moderately (Auger et al., 2002; Chiva-Blanch et al., 2013; Lucarini et al., 2021). Previous studies administered 3 mL/kg of red wine to rats on a high-fat diet (HFD), resulting in a 37.89 % reduction in triglyceride (TG) and a 26.4 % reduction in total cholesterol (TC) levels after 6 weeks, confirming its efficacy in lowering lipid levels (Mucaca et al., 2022). The intricate connection between lipid metabolism and redox homeostasis is notable. The NF-E2-related factor 2 (Nrf2) transcription factor serves as a primary regulator of antioxidation and environmental stress response. Activation prompts Nrf2 to translocate to the nucleus, stimulating the transcription of superoxide dismutase (SOD) and heme oxygenase (HO-1), bolstering resistance against oxidative stress (Mehrotra et al., 2014). Zhang et al. (2020) found that noni wine primarily influenced antioxidant biomarkers by modulating the Nrf2/glycogen synthase kinase-3 beta (Nrf2/Gsk-3β) pathway, thus modulating the oxidative stress response. Recent research increasingly underscores the crucial role of gut microbiota in lipid metabolism control. Cheng et al. (2023) highlighted Cabernet Sauvignon wine's ability to inhibit disruption of intestinal microflora caused by an HFD, boosting the presence of beneficial bacteria such as *Bacteroides*, *Verrucomicrobiota*, and *Akkermansiaceae*. Consequently, moderate wine consumption stands as a viable option for obesity prevention. However, the presence of sulfur dioxide (SO_2), an antioxidant used in wine production, may have adverse health effects, including headaches, diarrhea, rashes, and bronchoconstriction (Lisanti

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et al., 2019; Vally et al., 2009).

In recent years, significant efforts have been made to create healthier wines, primarily aimed at reducing the use of SO_2 due to its associated health risks. Polyphenols have emerged as potential substitutes for SO_2, supported by multiple academic validations indicating their positive impact on wine quality (Esparza et al., 2020; Gancel et al., 2021; Wang et al., 2019). In our previous study, the addition of 200 mg/L of resveratrol not only preserved the wine's inherent characteristics but also increased its volatile aromatic compounds (Ma et al., 2023). However, existing research predominantly focuses on assessing sulfur dioxide-free wine's quality, overlooking its potential health attributes. While polyphenols have shown promise in mitigating lipid disorders by influencing human metabolism and microbial ecology (Ma et al., 2020; Yu et al., 2016), scant attention has been paid to examining these health aspects concerning sulfur dioxide-free wine. Based on this premise, we hypothesize that sulfur dioxide-free wine may significantly contribute to lowering blood cholesterol levels by modulating oxidative stress and gut bacteria.

Therefore, this study extensively delved into the effects of wine enriched with dihydromyricetin, resveratrol, and tea polyphenols on oxidative stress, lipid metabolism, and intestinal microbiota in ICR mice subjected to an HFD. The research employed transcriptome analysis, western blotting, and high-throughput microbial sequencing. A comprehensive investigation was conducted to elucidate the regulatory potential and underlying mechanisms of sulfur dioxide-free wine on lipid metabolism.

2. Materials and methods

2.1. Wine making

Cabernet Sauvignon grapes were picked at Shuanglong Winery in Huailai, China (114 28′–115 10′ E, 40 22′–41.03′ N). Wine brewing process was conducted using our previous research reports. The entire winemaking process was performed in the winery of the Engineering Technology Research Center of Special Grain for Wine Making where alcoholic and malolactic fermentations were carried out at a controlled temperature (25 ℃). The previously described conditions are used in the wine brewing process (Ma et al., 2023). Treatments with different concentrations of polyphenol supplementation are described below:

S0: the addition of 70 mg/L SO_2; D0: the addition of 200 mg/L dihydromyricetin;
R0: the addition of 200 mg/L resveratrol; T0: the addition of 200 mg/L tea polyphenols.

2.2. Animal experiment

Sixty clean-grade ICR male mice were obtained from Chengdu Dashuo Experimental Animal Co., Ltd (Chengdu, China), weighted 20–22 g. All mice were housed in four per cage at 23 ± 2 ℃ with a 12-h light/dark cycle and free access to food and water in specific pathogen free experimental animal room in Testing Dog (Chengdu) Experimental Testing Co., Ltd. After the adaptation period, ICR mice were randomly divided into six groups (n = 10 per group): (1) NC group: supplemented with normal saline (20 mL/kg bd. wt); (2) AC group: supplemented with EtOH with the same alcohol content as wine (20 mL/kg bd. wt), EtOH concentrations were determined using a portable alcoholmeter; (3) S group: supplemented with the S0 group wine (20 mL/kg bd. wt); (4) D group: supplemented with the D0 group wine (20 mL/kg bd. wt); (5) R group: supplemented with the R0 group wine (20 mL/kg bd. wt); (6) T group: supplemented with the T0 group wine (20 mL/kg bd. wt). 20 mL/kg bd. wt. The body weight of each mouse was measured weekly. All the mice were fed the HFD (45 % energy from fat). 20 mL/kg bd. wt wine, being equivalent to drinking 150 mL of wine per day for an adult human weighing 60 kg. The National Institute of Alcohol Abuse and Alcoholism defines "moderatealcohol consumption" to be less than two drinks (28 g) per day for men. Therefore, it is safe for adults to drink 150 mL of wine on a daily basis, consuming an equivalent of 13 g of alcohol (McHenry et al., 2018). High fat feed was produced by Chengdu Dashuo Experimental Animal Co., Ltd (Chengdu, China). The detailed feed formulations are shown in Table S1. Animal weight and feed intake were monitored regularly and recorded. After 8-week period of experimental diet feeding, all mice were euthanized, then fasting blood was collected. Serum samples were obtained after centrifugation at 2000 rpm for 15 min. The liver was obtained and weighed immediately after dissection, and the remaining liver tissue was snap frozen in liquid nitrogen for 30 s and stored at −80 ℃ until further analysis. Animal experiments were performed according to the relevant guidelines of SPF Biotechnology Co., Ltd. The Committee on Care and Use of Laboratory Animals of the Frontier and Basic Research Institute of University of Electronic Science and Technology approved the experimental protocol (106142023081726451).

2.3. Serum and liver lipid assays

Serum and liver total cholesterol (TC), triglyceride (TG), low-density lipoprotein (LDL), and highdensity lipoprotein (HDL) contents were examined using the corresponding commercial enzymatic colorimetric assay kits (Jingmei Biotechnology Co., Ltd, Jiangsu, China) according to the manufacturer's instructions.

2.4. Serum and liver redox homeostasis

Serum and liver total antioxidation (*T*-AOC), reactive oxygen species (ROS), malondialdehyde (MDA), superoxide dismutase (SOD), Catalase (CAT) and glutathione peroxidase (GSH-Px) contents were determined using the corresponding commercial kits (Jingmei Biotechnology Co., Ltd, Jiangsu, China) according to the manufacturer's instructions.

2.5. Histological analysis

The fresh samples of liver were fixed in 4 % paraformaldehyde at 4 ℃ overnight, cut into 5 μm thick section. The sections were stained with hematoxylin and eosin (H&E), and then visualized by an optical microscope (Olympus, Japan) equipped with a digital camera (Hamamatsu, Japan).

2.6. Quantitative real-time PCR

Quantitative PCR analysis was performed according to the method described by Ma et al., with minor modifications (Ma et al., 2020). The liver was collected and snap-frozen using liquid nitrogen and then stored at −80 °C for further experiments. Total RNA from liver tissues was extracted by TRIzol reagent (Ambion, CA, USA). Next, RNA was converted to complementary DNA (cDNA) using a Fist-strand cDNA Synthesis Kit (Foregene, China). RT-qPCR was performed using RealStar Green Fast Mixture (Genstar, China) in CFX Connect Real-time System (BIO-RAD, Singapore) with the thermal cycle conditions: 1 cycle at 95 ℃ for 2 min; 40 cycles at 95 ℃ for 15 s, then at 58 ℃ for 30 s and at 72 ℃ for 30 s. Using β-actin as an internal reference, quantitative changes of gene expression were calculated by the delta-delta-Ct. The list of the primers used in this study is summarized in Table S2.

2.7. Western bolt analysis

The western blot analysis was performed as previously described (Yu et al., 2016). Total proteins were extracted from the liver tissues with 400 μL cold RIPA buffer (Genstar, Beijing, China), 4 μL Protease Cocktail (YEASEN, Shanghai, China). The protein concentration was measured using BCA assay kit (Beyotime, Shanghai, China). Equal amounts of protein (60 μg per lane) were electrophoresed by 10 % sodium dodecyl

sulfate (SDS)–polyacrylamide gel electrophoresis, then transferred onto PVDF membranes. After blocking with 5 % no fat milk powder, the membranes were incubated with primary antibodies at 4 ∘C overnight. After washing procedure using TBST, the membranes were incubated with secondary antibodies for 1 h at room temperature. The protein bands were visualized using enhanced chemiluminescence (ECL) detection kits (Tanon, Shanghai, China). The bands were analyzed using the ImageJ software and the expression ratios were normalized to the expression ratio of β-actin. The sources of purchase, catalog numbers, and dilution multiples for the antibodies utilized in this study are listed in Table S3.

2.8. Gut microbiota assessment

Six mice were randomly selected from each group for gut microbiota analysis. Microbial genomic DNA was extracted from mouse colonic feces using the NucleoSpin 96 Soil, 96-well kit for DNA from soil (Macherey-nagel, Germany) according to the manufacturer's instructions. The hypervariable V3-V4 region of the bacterial 16S rRNA gene was analyzed. All DNA samples were amplified and purification using primers pair (338F and 806R). Finally, the high-throughput sequencing was performed on the Illumina HiSeq platform by HiSeq2500 PE250 (Illumina, USA). The original data obtained by sequencing were spliced and filtered to get the effective data. Then the taxonomic analysis of operational taxonomic units (OTUs) aggregation and species was carried out. Sequences were clustered into the same OTUs based on the similarity of greater than 97 % (USEARCH, version10.0).

2.9. Statistical analysis

All data are expressed as the mean ± standard error of the mean (SEM). The statistical analyses were performed using SPSS 20.0 software (SPSS IBM, Inc., Armonk, NY, USA), and means were considered statistically significant and highly significant at $p < 0.05$.

3. Results

3.1. Body weight and organ index

At the start of the experiments, all animals had similar body weights. Following an 8-week duration, the experimental groups (D, R, S, and T) demonstrated significantly lower body weight gain than the two control groups (NC and AC). Furthermore, the experimental groups exhibited significantly lower liver organ indices, with group R showing the lowest liver organ index (Fig. 1).

3.2. Serum lipid and oxidative stress levels

The experimental groups (D, R, S, and T) displayed a significant decrease in serum TC, TG, and high-density lipoprotein (HDL) levels compared with the control groups (NC and AC) ($p < 0.05$). Among these groups, group D exhibited the lowest TC level (1.11 mmol/L), while group R exhibited the lowest TG and HDL levels (1.32 mmol/L and 0.32 mmol/L, respectively). Notably, groups D and R exhibited even lower TC and TG levels than group S ($p < 0.05$). Conversely, no significant differences in low-density lipoprotein (LDL) levels were observed among the six groups ($p > 0.05$). In terms of blood antioxidant markers, all experimental groups demonstrated increases in total antioxidant capacity (*T*-AOC) and SOD levels, coupled with a decrease in reactive oxygen species (ROS) levels. Group D exhibited the highest *T*-AOC (72.39 pg/mL) and lowest ROS (1462.89 pg/mL) levels. Furthermore, group R exhibited the highest catalase (CAT) level (3087.13 pg/mL), which was significantly higher than that in both control groups ($p > 0.05$). However, plasma glutathione peroxidase (GSH-Px) levels did not differ significantly among the groups ($p > 0.05$) (Table S4).

3.3. Liver lipids and oxidative stress levels

The experimental groups (D, R, S, and T) exhibited significant decreases in serum TC, TG, and HDL levels compared with the control groups (NC and AC) ($p < 0.05$) (Fig. 2-a, b, c). Notably, group R yielded the lowest TG and HDL values. However, there were no significant differences in liver LDL content among the groups (Fig. 2-d). These findings are consistent with the regulation of blood lipid levels. For a detailed examination of liver samples from the experimental groups, tissue sections were subjected to H&E staining, and representative images are depicted in Fig. 2-e. In the NC and AC groups, HFD led to increased microbubble fatty degeneration around the central vein of the liver (indicated by circles). Conversely, the experimental groups (D, R, S, and T) supplemented with wine showed a distinct reduction in this condition, suggesting that moderate wine consumption effectively mitigates HFD-induced fatty degeneration of the liver. The pattern observed in the liver antioxidant index resembled that of the blood antioxidant index. The experimental groups exhibited increased *T*-AOC and SOD levels alongside decreased ROS levels (Fig. 2-f, g, h). Moreover, these groups exhibited higher CAT content and reduced malondialdehyde (MDA) levels (Fig. 2-j, k). However, GSH-Px levels did not significantly differ among the groups ($p > 0.05$) (Fig. 2-i).

3.4. Association of liver oxidative stress and fat metabolism with gene expression and protein levels

Based on the comprehensive evaluations conducted previously, we believe that group R within the experimental set might effectively regulate lipid metabolism. To delve deeper into the mechanisms

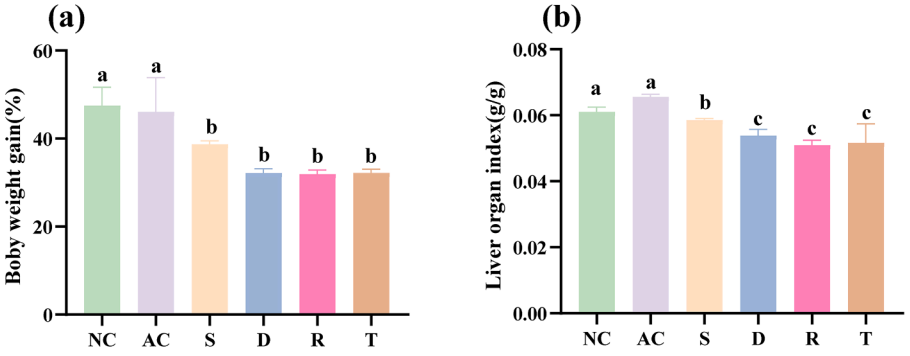

Fig. 1. a- Body weight gain index of mice in different groups; b- liver organ index of different groups of mice. Different superscript letters (a–c) for the same parameter denote significant differences ($p < 0.05$).

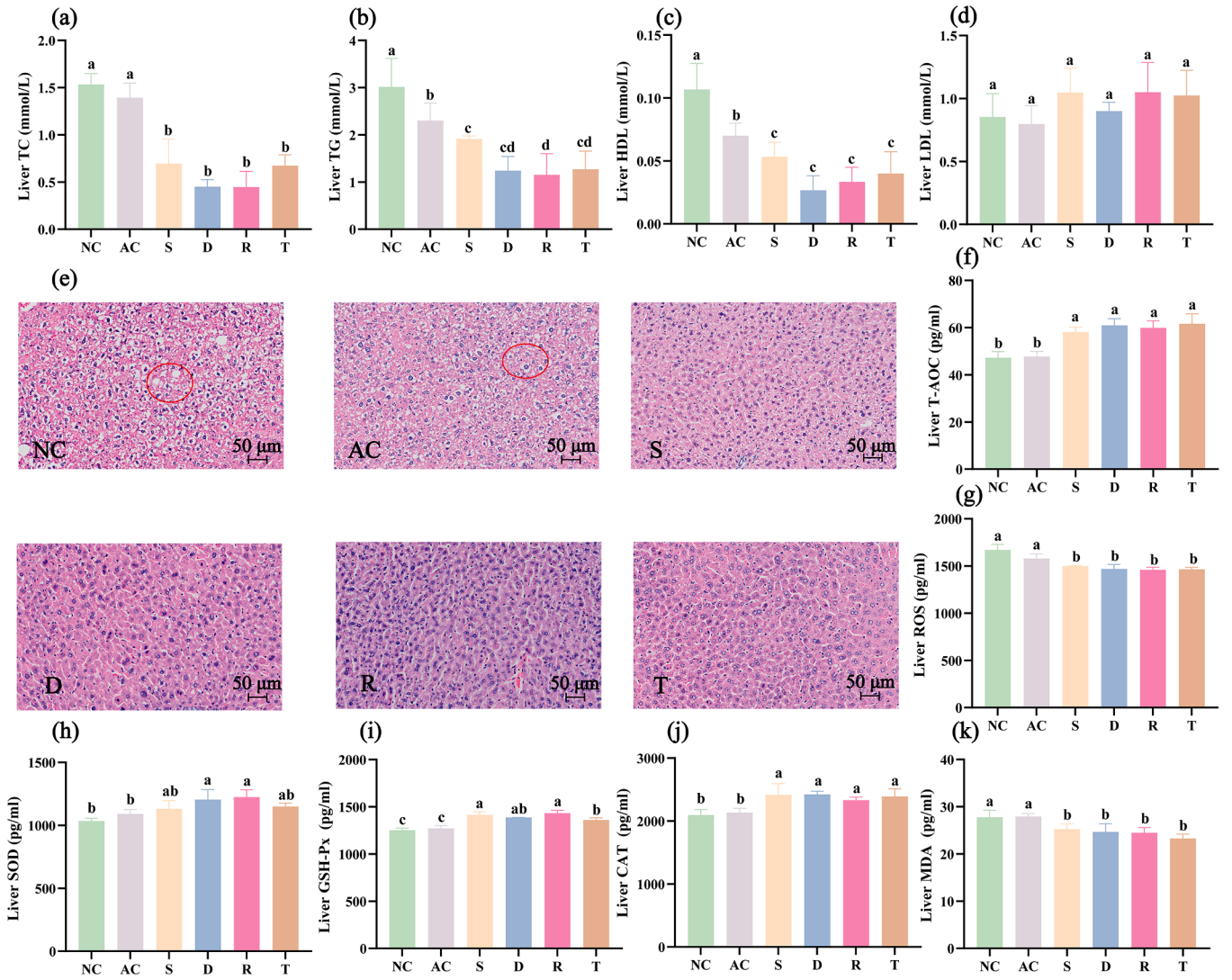

Fig. 2. Liver lipid and antioxidant indices in various mouse. (a) Liver TC level. (b) Liver TG level. (c) Liver HDL level. (d) Liver LDL level. (e) H&E staining of Liver (200×). (f) Liver *T*-AOC level. (g) Liver ROS level. (h) Liver SOD level. (i) Liver GSH-Px level. (j) Liver CAT level. (k) Liver MDA level. Different superscript letters (a–b) for the same parameter denote significant differences ($p < 0.05$).

underlying the effect of the R group on lipid metabolism, further investigations will be pursued. In this pursuit, gene expression and protein levels of Nrf2, HO-1, and NAD(P)H quinone dehydrogenase 1 (NQO1) in the liver were analyzed to determine their impact on the oxidative stress mechanism. Compared with the control groups (NC and AC), the R group exhibited a significant increase in the relative expression levels of *Nrf2*, *HO-1*, and *NQO1*, which corresponded to their protein level measurements. Additionally, the S group demonstrated enhanced *NQO1* expression compared with the NC group, with a similar trend observed in the AC group, albeit without significant advantages in protein level detection (Fig. 3a–f, k). Regarding lipid metabolism, this study analyzed the gene expression and protein levels of carnitine palmitoyltransferase I (*Cpt-1*) and peroxisome proliferator-activated receptor alpha (*Pparα*). The findings indicated a significant upregulation of *Cpt-1* and *Pparα* levels in the R group, which were significantly higher than those in the other three groups. These observed patterns were also evident in the protein analyses (Fig. 3g–k).

3.5. Changes in the integral structure of the gut microbiota

MiSeq sequencing was performed using cecal contents to explore the impact of different treatments on the gut microbiota composition of HFD-fed ICR mice. A total of 241 operational taxonomic units (OTUs) were identified across all samples, with specific OTUs of 125, 140, 155, and 164 identified in the NC, AC, S, and R groups, respectively. Of these, 76 OTUs were shared among all four groups, with additional unique OTUs identified in each group (13, 20, 23, and 10 for NC, AC, S, and R groups, respectively) (Fig. 4-a). This study investigated the effects of various treatments on the alpha diversity (α-diversity) (Shannon and Chao indices) and beta diversity (β-diversity) of the gut microbiota at the OTU level. Both the S and R groups exhibited an increasing trend in both Chao and Shannon indices, contrasting with the NC and AC groups. Notably, the R group exhibited significantly higher Chao ($p < 0.05$) and Shannon ($p < 0.01$) indices compared with the AC group, suggesting that wine from the R group effectively enhanced the diversity of gut microbial species in HFD-fed mice (Fig. 4-b).

Weighted UniFrac analysis, along with principal coordinate (PCoA) and nonmetric multidimensional scaling (NMDS) analyses, were utilized to evaluate alterations in the gut microbiota compositions of the NC, AC, S, and R groups compared with those of the control group (Fig. 6). These analyses highlighted significant alterations in the gut microbiota composition following wine consumption. PCoA revealed that the first principal coordinate (PC1) explained 42.10 % of the overall variation, while PC2 accounted for 28.75 % of the microbial community variability

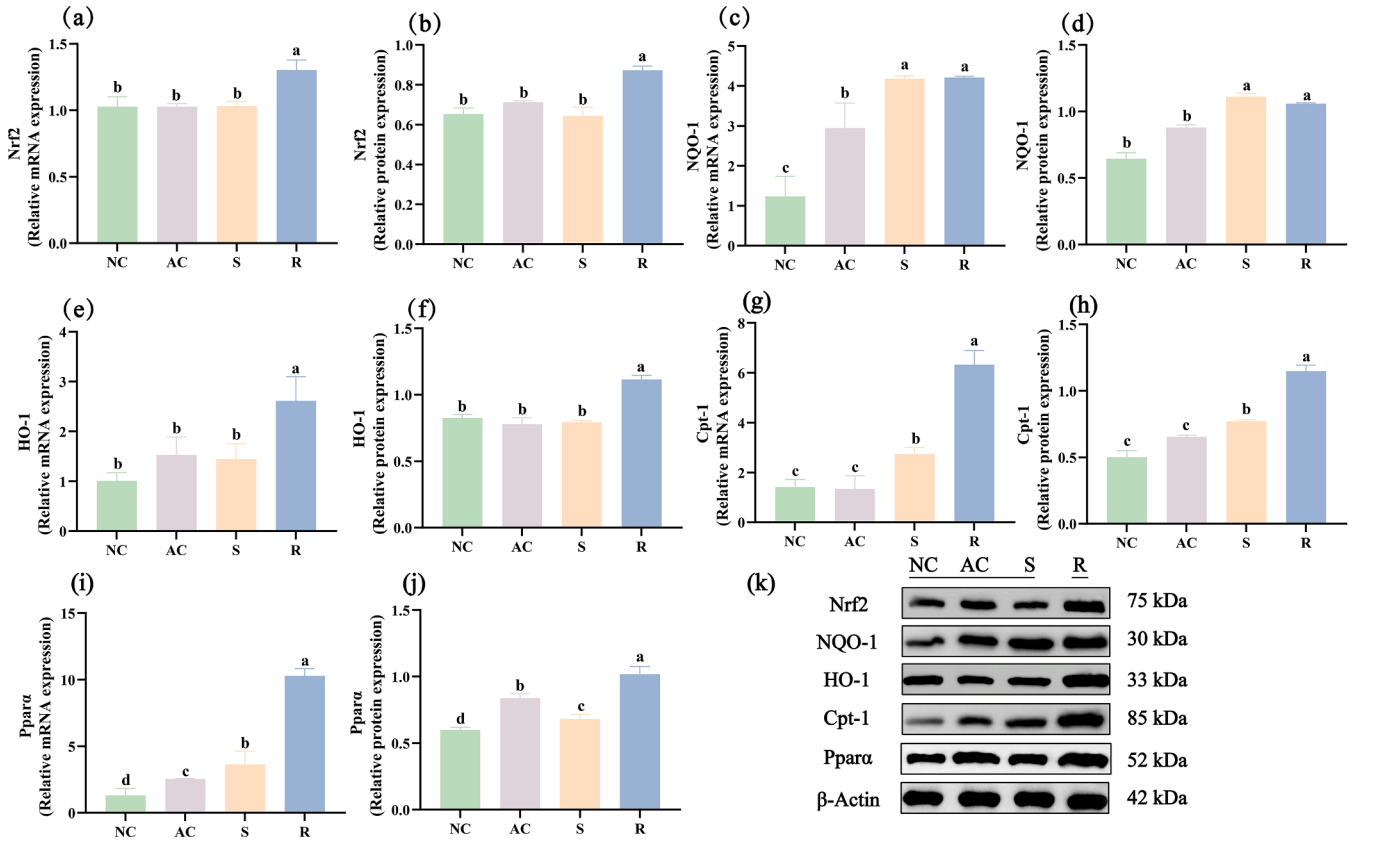

Fig. 3. Analyses of mRNA expression and protein levels of some oxidative stress and fat metabolism factors in the liver. (a) Nrf2 mRNA relative expression. (b) Nrf2 protein level. (c) NQO-1 mRNA relative expression. (d) NQO-1 protein level. (e) HO-1 mRNA relative expression. (f) HO-1 protein level. (g) Cpt-1 mRNA relative expression. (h) Cpt-1 protein level. (i) Pparα mRNA relative expression. (j) Pparα protein level. (k) Expression of related proteins in liver. Different superscript letters (a–d) for the same parameter denote significant differences ($p < 0.05$).

(Fig. 4-c). Both NMDS and PCoA analyses illustrated a notable similarity between the S and R groups, distinctly separated from the AC and NC groups (Fig. 4-d).

3.6. Key taxonomic species analysis

At the phylum level of microbial composition, the predominant phyla were Firmicutes, Actinobacteria, and Proteobacteria. In the NC group, Firmicutes and Actinobacteria were most prevalent, accounting for 72.29 % and 22.53 %, respectively, whereas they were least abundant in the AC group. Conversely, Proteobacteria exhibited the highest relative abundance in the AC group (16.19 %) (Fig. 4-e). Additionally, the Firmicutes/ Bacteroidetes (F/B) ratio in the S and R groups was significantly lower than that in the AC and NC groups ($p < 0.05$). The R group displayed the lowest F/B ratio, although no significant difference was observed compared with the S group (Fig. 5-f). At the genus level, the dominant genera included *Lactobacillus*, *Allobaculum*, Turicibacter, *Bifidobacterium*, and *Adlercreutzia*. *Lactobacillus* exhibited the highest relative abundance in the NC group (46.16 %) and the lowest relative abundance in the AC and S groups (12.31 % and 13.00 %, respectively). *Allobaculum* was predominantly found in the NC, S, and R groups, with minimal presence in the AC group. *Turicibacter* was primarily detected in the S and R groups, with minimal presence in the NC group. *Bifidobacterium* exhibited the highest relative abundance in the NC group (17.98 %) and was almost absent in the S group (Fig. 5-g).

The linear discriminant analysis effect size represents a robust tool used to identify and interpret biomarkers within high-dimensional biological data. It facilitates comparisons among multiple groups, highlighting both statistical significance and biological relevance to identify biomarkers exhibiting significant differences between these groups. This analysis focuses on statistically distinct biomarkers among diverse groups. The distribution bar plot of linear discriminant analysis (LDA) values demonstrates that values exceeding 2 indicate species with significant differences, serving as statistically significant biomarkers. This plot displays species exhibiting significantly different abundances among diverse groups, with the lengths of the bars indicating the magnitude of their impact. A total of 30 species with LDA values >2 were identified, with the R group comprising the highest number of 15 species, followed by the S group with 8 species, the AC group with 4, and the NC group with 3. These identified species primarily belonged to the Actinobacteria, Firmicutes, and Proteobacteria phyla. At the genus level, 15 species were annotated, including *Staphylococcus*, *Psychrobacter*, *Roseomonas*, *Corynebacterium*, *Megasphaera*, *Facklamia*, *Bilophila*, and *Oleibacter*, among others. These microbial species potentially serve as biomarkers, aiding in distinguishing different groups based on dissimilarities in abundance (Fig. 5-h, i).

The heatmap visualization illustrated correlation coefficients between bacterial abundances from the phylum to family levels alongside serum lipid and oxidative stress indicators (Fig. 5). Notably, the relative abundances of specific taxa—Pseudomonadales, Actinomycetales, *Moraxellaceae*, *Corynebacteriaceae*, *Aerococcaceae*, *Brucellaceae*, *Porphyromonadaceae*, *Acetobacteraceae*, and *Oleiphilaceae*—exhibited negative correlations with TG, TC, and HDL levels while positively correlating with the F/B ratio. Additionally, *Streptococcaceae* displayed a positive correlation with TG and TC levels, while LDL levels exhibited positive correlations with the relative abundances of Erysipelotrichia, Erysipelotrichales, *Erysipelotrichaceae*, *Chitinophagaceae*, and *Halomonadaceae*, and a negative correlation with the relative abundance of *Cyanobacteria*. Moreover, positive correlations were observed between *T*-AOC, CAT, and SOD levels and the relative abundances of Pseudomonadales,

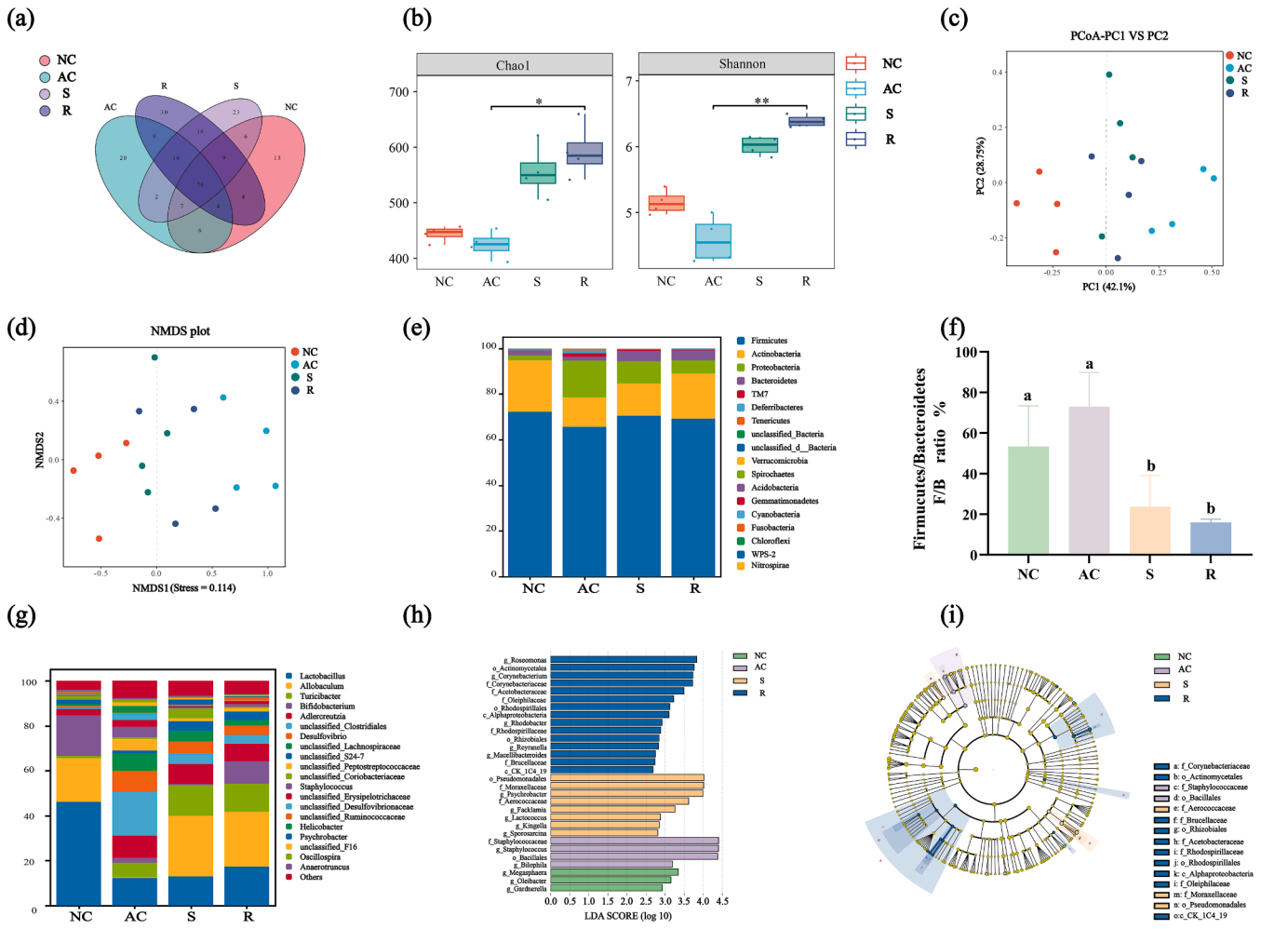

Fig. 4. The effect of sulfur dioxide-free wine on the composition of gut microbiota in HFD mice. (a) Venn diagram. (b) Chao1 index and Shannon index (* $p < 0.05$ and ** $p < 0.01$). (c) PCoA based on relative abundances of all bacterial strains using weighted UniFrac metric (d) NMDS based on relative abundances of all bacterial strains using weighted UniFrac metric (e) The relative abundances at the phylum level. (f) F/B ratio (g) The relative abundances at the genus level. (h) Linear discriminant analysis (LDA) scores. (i) The length of the bar represents the LDA score.

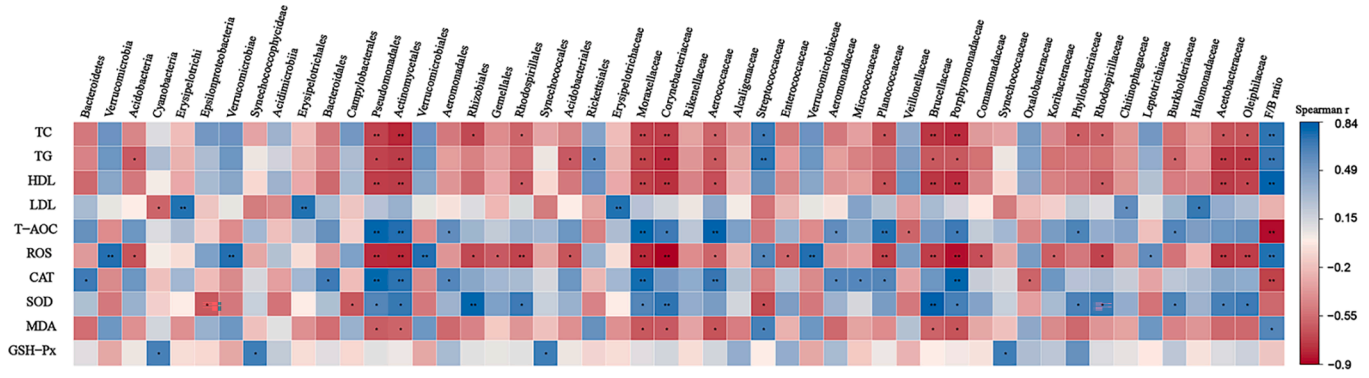

Fig. 5. Correlation analysis between gut microbiota and Antioxidant markers and blood lipid indexes * $p < 0.05$ and ** $p < 0.01$ (n = 4).

Actinomycetales, *Moraxellaceae*, and *Porphyromonadaceae*. Conversely, negative correlations were observed between ROS and MDA levels and the relative abundances of Pseudomonadales, Actinomycetales, *Moraxellaceae*, and *Porphyromonadaceae*. Interestingly, the F/B ratio showed negative correlations with *T*-AOC and CAT levels but displayed positive correlations with ROS and MDA levels. Lastly, positive correlations were observed between GSH-Px levels and *Cyanobacteria*, *Synechococcophycideae*, Synechococcales, and *Synechococcus*.

4. Discussion

Obesity has emerged as a significant risk factor for various metabolic conditions. Moderate wine consumption has shown promise in regulating lipid metabolism and mitigating adiposity. However, the development of wine as a functional product has faced challenges due to adverse reactions linked to the commonly used antioxidant SO_2. Numerous studies have indicated that polyphenols possess the potential

to act as viable substitutes for SO_2. This study sheds light on sulfur dioxide-free wine, enriched with polyphenols as antioxidants, demonstrating its superior efficacy in preventing HFD-induced lipid metabolism disorders. The potential mechanism behind this effectiveness could be attributed to sulfur dioxide-free wine's capacity to mitigate HFD-induced oxidative stress while also influencing the regulation of gut microbiota (Fig. 6).

ROS are potent oxidizing agents generated during oxygen metabolism, influencing the onset of oxidative stress. Normally, the body maintains a balance between ROS production and elimination, managed by enzymatic antioxidants (e.g., SOD, CAT, GSH-Px) and non-enzymatic antioxidants (e.g., vitamin C, glutathione) (Valko et al., 2007). However, disruptions in energy intake and metabolism can upset this equilibrium, causing an abnormal ROS accumulation and fostering oxidative stress. Oxidative stress triggers endothelial cells to release cytotoxic selectins and endothelial particles, disturbing their regular function (Lacroix et al., 2012). Endothelial cells play a crucial role in regulating fatty acid utilization, the primary source of energy for the human body. Under oxidative stress, this regulatory capacity diminishes, leading to abnormal fat accumulation. Key genes such as *NQO1* and *HO1*, regulated by the Nrf2 signal, are pivotal in combating oxidative stress (Mehrotra et al., 2014). The Nrf2–HO1 pathway, a significant endogenous antioxidant system, reduces ROS-induced oxidative stress (Vasileva et al., 2020). After Nrf2 is activated, it migrates to the nucleus and induces SOD and HO-1 transcription. As a primary antioxidant, bilirubin, a product of HO-1 breakdown, can effectively prevent unbound heme from causing oxidation, thereby enhancing microcirculation (Wang & Yuan, 2019). SOD and CAT act as the first line of defense against oxidative injury by reducing ROS (Kitada et al., 2011). MDA production and lipid peroxidation are closely linked. Unsaturated fatty acids become susceptible to oxidation by oxygen free radicals, resulting in lipid oxidation and MDA production and production of other byproducts (Henkel et al., 2018). Our study highlighted that sulfur dioxide-free wine consumption notably increased transcription and protein levels of *Nrf2*, *HO-1*, and *NQO1* in the liver. It also raised serum and liver SOD and CAT levels while reducing ROS and MDA levels. The observed pattern aligns with the findings of Zhang et al. (2020), who ascribed the antioxidant modulation of wine to its polyphenolic content. Loke et al. (2010) found a positive correlation between total phenol, anthocyanin, and catechin levels in wine and antioxidant activity. Micallef et al. (2007) observed that consumption of 400 mL of red wine daily for 2 weeks substantially enhanced the total antioxidant status of plasma ($p < 0.03$) and reduced circulatory oxidative stress. Compared with traditional wines treated with SO_2, our research indicates that consumption of sulfur dioxide-free wines fermented with resveratrol has a greater effect on the *Nrf2* gene. Baker et al. (2013) demonstrated that resveratrol, glycyrrhizin, and omega-3 polyunsaturated fatty acids are Nrf2 activators, reinforcing our findings that resveratrol-containing sulfur dioxide-free wine positively impacts the Nrf2 pathway, further demonstrating that this wine exhibited greater resistance to oxidative stress.

In this study, the experimental group consuming wine showed substantial reductions in weight gain, liver index, as well as in blood and liver levels of TG, TC, and HDL of mice, with no observed change in LDL levels. These outcomes suggest a potential contribution of polyphenols to reducing adiposity, aligning with a previous study demonstrating reduced visceral adiposity in mice supplemented with grape polyphenols (Rivers et al., 2018). The observed changes in blood lipid levels might be attributed to enhanced liver fat metabolism and accelerated fatty acid oxidation. PPARα, a pivotal fatty acid receptor, serves as the primary transcription factor for the fatty acid oxidase gene and plays a crucial role in maintaining physiological lipid and energy metabolism. It regulates lipoprotein metabolism, impedes fatty acid metabolism, and enhances fatty acid transport and utilization in the liver. CPT-1 is an enzyme that controls the rate of fatty acid oxidation (Wang et al., 2020). This study's findings support the ability of resveratrol-fortified sulfur dioxide-free wine to stimulate the *Pparα* and *Cpt-1* genes. Interestingly, the AC group exhibited increased PPARα protein levels, although, not statistically significant. Notably, the hepatic levels of TC and TG in the AC group were lower than those in the NC group. Previous research has demonstrated that ethanol's impact on the gastrointestinal tract and peritoneum leads to systemic stress and disruption of lipid metabolism (Baraona & Lieber, 1979). Studies by Justice et al. (2019) demonstrated that intermittent voluntary consumption of 20 % ethanol in Wistar rats for 3 months resulted in decreased epididymal fat, blood sugar, non-HDL cholesterol, and TC levels. These effects are accompanied by reduced expression of 3-hydroxy-3-methylglutaryl coenzyme A reductase, sterol regulatory element-binding protein 2, cyclooxygenase-2, and v-rel avian reticuloendotheliosis viral oncogene homolog A, indicating the down-regulation of genes associated with cholesterol synthesis and inflammation, mirroring the outcomes of this experiment. Previous research has demonstrated that phenols inhibit pancreatic lipase, increase fecal TG excretion, and directly reduce intestinal fat absorption (Mcdougall & Stewart, 2005). Additionally, increased activity and energy consumption tend to boost adipose metabolism. While wine consumption significantly increases oxygen consumption in obese rodents (Teske et al., 2012), this study did not detect changes in oxygen consumption or fecal fat in the rodents used.

The gut microbiota has emerged as a pivotal player in lipid- and health-related research (Fischbach, 2018). Dysbiosis of the gut microbiome is strongly correlated with pathophysiological traits observed in obesity, metabolic syndrome, and conditions such as nonalcoholic fatty liver disease (Del Chierico et al., 2017). HFDs substantially alter the composition and function of the intestinal microflora, impacting the host's metabolism (Kolodziejczyk et al., 2019). In our study, oral

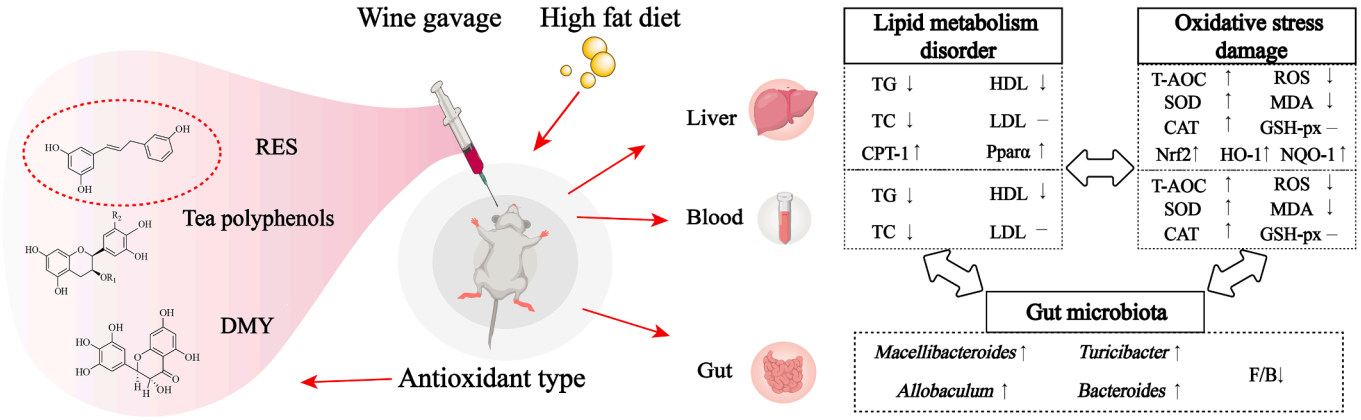

Fig. 6. A schematic representation demonstrating sulfur dioxide-free wine with polyphenols' effects on lipid metabolism, oxidative stress, and gut microbiota in HFD mice and its mechanism.

administration of sulfur dioxide-free wine demonstrated a regulatory effect on gut dysbiosis, notably improving the α- and β-diversity of the gut microbiota. We found that sulfur dioxide-free wine increased the abundance of key microbiota associated with enhanced lipid metabolism and antioxidant activity. Specifically, the relative abundances of *Bacteroides* and *Allobaculum* significantly increased in the S and R groups. This trend aligns with findings from Santacruz et al. (2010) who observed decreased *Bifidobacterium* and *Bacteroides* counts in overweight 24-week-pregnant women. Emoto et al. (2017) replicated a comparable rule in their study on cardiovascular patients. Angiopoietin-like protein 4 (ANGPTL4), also known as fasting-inducible factor, plays a role in lipid metabolism by inhibiting lipoprotein lipase, a crucial enzyme in lipid deposition. In a separate study by Zheng et al. (2021), a positive correlation was found between the abundance of *Allobaculum* in mice with high lipid content and ANGPTL4 expression. The significance of the F/B ratio in various chronic metabolic diseases cannot be understated. Previous research has shown an increase in the F/B ratio alongside a decrease in *Bacteroides* abundance—specifically, *Bacteroides vulgaris* and *Bacteroides dorei*—in individuals with obesity and cardiovascular disease (Li et al., 2022). Interestingly, in this study, orally consuming sulfur dioxide-free wine reversed the increasing trend of the F/B ratio in HFD-fed rodents. Beyond genetic factors, the influence of intestinal microorganisms on intestinal metabolites significantly impacts lipid metabolism. Dysregulation in bile acid metabolism leads to insufficient activation of bile acid receptors such as farnesoid X receptor and Takeda-G-protein-receptor-5, contributing to decreased energy expenditure and increased adipose production. Lynch et al. (2023) highlighted *Turicibacter*'s ability to alter host genes related to bile acid and lipid metabolism, reducing cholesterol and triglyceride levels in rodents. In our study, the S and R groups displayed substantially higher relative abundance of *Turicibacter* than the NC and AC groups. Moreover, symbiotic bacterial metabolites, especially short-chain fatty acids, such as acetic, propionic, and butyric acids, play a role in regulating the intestinal microflora and host's inflammatory response. The presence of *Macellibacteroides* showed a positive correlation with the production of short-chain fatty acids (Ai et al., 2022). *Macellibacteroides* was the most distinctive microorganism solely present in the R group. This suggests that the regulation of intestinal metabolites could be one of the mechanisms involved in lipid regulation in this experiment. However, it is important to know that this aspect fell beyond the scope of our study.

5. Conclusion

This study examined the impact of wine enriched with antioxidants—resveratrol, dihydromyricetin, and tea polyphenols—on lipid metabolism in mice. The findings demonstrate that moderate consumption of these polyphenol-rich wines can effectively prevent HFD-induced oxidative stress and fat accumulation. Notably, wine enriched with polyphenols, particularly resveratrol, showed a stronger effect than traditional SO_2-treated wine. The mechanism underlying the effect of these polyphenols was further investigated. The results indicate that the mechanism primarily involves the regulation of key signaling factors in the liver—Nrf2, HO-1, CPT-1, and Pparα—alongside the proliferation of key gut microorganisms, such as *Macellibacteroides*, *Turicibacter*, *Allobaculum*, and *Bacteroides*. Overall, these results highlight that moderate consumption of wine enriched with polyphenols as antioxidants could serve as an effective strategy in preventing obesity. However, further research investigating the effects of this sulfur dioxide-free wine on gut metabolism and barrier function is warranted.

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CRediT authorship contribution statement

Yi Ma: Funding acquisition, Supervision. **Kangjie Yu:** Writing – original draft, Writing – review & editing. **Ning Wang:** Methodology. **Xiongjun Xiao:** Formal analysis. **Yinjiang Leng:** Investigation. **Jun Fan:** Resources. **Yong Du:** Resources. **Shuanghui Wang:** Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2023.101079.

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