EFFECT OF BAICALIN ON THE INTESTINAL MICROBIOTA IN OBESE MICE INDUCED BY HIGH-FAT DIET

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Abstract. In order to analyze the effect of baicalin on intestinal microbiota (IM) of obese mice induced by high-fat diet (HFD), four groups of mice, i.e., normal control group, HFD group, baicalin-treated group of normal control, and baicalin-treated group of HFD, were set up. After 12 weeks of treatment, the mice's body weight, insulin sensitivity, liver histopathological analysis and immunohistochemical analysis were measured. Combining with high-throughput sequencing of IM 16S rRNA gene, the characteristics of obesity induced by HFD and the therapeutic effect of baicalin on obese mice induced by HFD were explored. HFD significantly increased mice body weight, insulin resistance, liver fat accumulation, and the number of macrophages and T cells in the liver, and significantly changed the structure of obese mice IM. Baicalin treatment significantly reduced the weight and insulin resistance of obese mice induced by HFD, liver fat accumulation, and the number of macrophages and T cells, and significantly changed the structure of IM. This study provided reference data for understanding the effect of baicalin on IM composition and metabolism in the treatment of obesity caused by HFD, and helped us to understand the effect of chemicals on IM and its potential effect on host metabolism.
Keywords: *body weight, blood glucose level, insulin resistance, metabolic syndrome, microbiota structure*

Introduction

Currently, approximately one-third of the world's adults are at risk of obesity-related metabolic syndrome such as non-alcoholic fatty liver disease (NAFLD), type 2 diabetes, and cardiovascular disease due to overweight (DeMarco et al., 2014; Polyzos et al., 2022). Obesity and metabolic syndromes caused by it have shown an explosive epidemic trend, becoming one of the most serious types of diseases threatening public health (Radu et al., 2023). Simultaneously, obesity is also an important cause of other liver diseases, such as liver fibrosis, cirrhosis, and primary hepatocellular carcinoma (Shah et al., 2023). Therefore, exploring the methods and mechanisms for the prevention and treatment of obesity and obesity-induced metabolic syndromes has important theoretical and clinical application value.

Obesity is a major cause of metabolic syndromes. Although the causes and mechanisms of obesity are complex and diverse, unbalanced diet is the main reason for disturbing normal energy metabolism, inducing excessive accumulation of lipids and eventually leading to obesity (Klaauw et al., 2015). Patients with obesity and metabolic syndrome are accompanied by low-level, systemic chronic inflammation, and the inflammatory response leads to impaired insulin action and metabolic abnormalities (Cani et al., 2007). Studies have confirmed that the activation of inflammatory effector molecules contributes to the desensitization of insulin signaling pathway (Cai et al., 2005). At the molecular level, the activation of IκB kinase complex, extracellular signal-regulated protein kinases 1 and 2 (ERK1/2), and c-Jun N-terminal kinases (JNKs) in the inflammatory tissues of obese individuals all reduce the tyrosine phosphorylation of insulin receptor substrate proteins, leading to the attenuation of insulin signaling (Tanti et al., 2013). Loss of insulin sensitivity triggers fasting hyperglycemia and increases hepatic lipid synthesis, dyslipidemia, hypertension, and fat accumulation in adipose tissues

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(Saltiel et al., 2001). However, excessive caloric intake, increased fat accumulation, and lipotoxicity also activate the production of effector molecules (cytokines) and cells mainly involved in innate immunity (Cani et al., 2007; Cai et al., 2005). These products enhance the chronic low-level inflammatory state and induce the recruitment and activation of a variety of mature immune cells (including mast cells, macrophages, and dendritic cells) and adipocytes in metabolic tissues, especially adipose tissues, thus further enhancing the inflammatory response (Lumeng et al., 2011).

Intestinal microbiota (IM) play an important role in many aspects of vertebrate growth, development, metabolism, and immunity (Bui et al., 2023; Seth et al., 2019). Studies have shown that IM play an important role in the occurrence and development of metabolic diseases such as obesity, insulin resistance, atherosclerosis, and NAFLD (Cani et al., 2007). IM assist in degradation of indigestible food components, such as polysaccharides in fermented diet (Qin et al., 2010), and regulate the host energy homeostasis by exchanging metabolites with the host and participating in the host signaling pathway to regulate bile acid, lipid, and amino acid metabolism, as well as the host gene expression (Velagapudi et al., 2010). The structural imbalance of IM caused by high-fat diet (HFD) intake may damage the intestinal barrier function, cause the increase of inflammatory factors and lipopolysaccharides levels in the circulatory system, thereby triggering metabolic inflammation and inducing insulin resistance, obesity and even diabetes (Li et al., 2022). The imbalance of IM causes the weakening of intestinal barrier function, and the pathogenic bacteria and endotoxin in the intestine enter the body through the intestinal mucosal barrier, which becomes an important initiating and sustaining factor for the persistence of inflammation and the induction of cancer (Liu et al., 2018).

Recently, in addition to probiotic therapy, traditional Chinese medicine, as an effective treatment method, provides new ways and methods for the prevention and treatment of obesity and metabolic syndromes caused by obesity (Zu et al., 2016). Baicalin is a flavonoid extracted from the dried root of *Scutellaria baicalensis* Georigi, which has a variety of pharmacological effects such as antihypertensive, hepatoprotective, antibacterial and anti-inflammatory effects (Zha et al., 2020). Baicalin improves inflammatory disorder and tissue damage by down-regulating the concentrations of a variety of cytokines and proteins such as tumor necrosis factor-α (TNF-α), immunoglobulin E (Ig E), interleukin-6 (IL-6) and interleukin-1β (IL-1β) (Yan et al., 2016; Dinda et al., 2017; Wu et al., 2019). Considering that host inflammation, immunity, oxidative stress response, insulin resistance and lipid metabolism disorders are closely related to IM, we speculate that baicalin can effectively prevent obesity and insulin resistance induced by HFD, and change the structure of IM in obese hosts. In this study, C56BL/6J male mice were used to investigate the therapeutic effect of baicalin on obesity induced by HFD, and to analyze the changes in the structure and metabolism of IM during this process.

Materials and methods

Laboratory animal feeding and sample collection

The experimental protocol was approved by the Animal Ethics Committee of Qilu Normal University and was performed according to its guidelines. Thirty-two 6-week-old C56BL/6J male mice purchased from the Experimental Animal Center of Shandong Academy of Medical Sciences (Jinan, Shandong Province) were randomly divided into 4 groups after 2 weeks of adaptive feeding, with 8 mice in each group. Normal chow

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diet (NCD) group was ad libitum fed RD D12450B diet with 10% fat energy (Research Diets, Inc., New Brunswick, NJ, USA). The HFD group was ad libitum fed with RD D12492 diet with 60% energy from fat (Research Diets, Inc., New Brunswick, NJ, USA). Baicalin (100 μg/kg body weight) was added to normal and HFD in the NCD and baicalin treatment group (NCDBCL), and HFD and baicalin treatment group (HFDBCL) according to previously described (Gao et al., 2022). Water intake was free during the experiment. The mice were maintained 12 weeks at 23 ± 1°C and 55 ± 5% humidity with 12-h light-dark switch. The body weight of mice in each group was measured at the same time every week during the experiment.

Insulin tolerance test (ITT) was performed in the last week of the experiment. The mice were fasted 8 h before the ITT experiment, and after intraperitoneal injection of insulin (0.75 U/kg body weight), their blood glucose levels (BGLs) were measured by glucose meter (Yuyue Medical Equipment Co., Ltd., Danyang, Jiangsu, China) at 0, 15, 30, 60, 90, 120 and 150 min (Li et al., 2022).

At the end of the experiment, at least three replicate fecal samples were collected from each group before dissection and stored at -80°C for subsequent sequencing analysis. The mouse cervical spine was then dislocated and fixed on the anatomical plate, the abdominal cavity was opened, and the liver was collected. After washing with phosphate buffer, the liver was fixed in 10% formalin fixative for histological analysis.

Histological analysis

Fixed liver tissue was embedded in paraffin, sectioned (at a thickness of 4 μm), and stained with oil red for fat after labeling the nucleus with hematoxylin-eosin. The distribution of macrophages in mouse liver was detected by F4/80 immunohistochemistry (Li et al., 2022). Tissue sections were photographed and observed using a BX53 + DP26 light microscope (Olympus, Japan) (Lin et al., 2019).

Analysis of IM composition

Total genomic DNA of IM was extracted using a strong fecal DNA extraction kit (QIAGEN, Germany) according to the manufacturer's instructions [22]. Quality of DNA was detected using a Nanodrop 2000 spectrophotometer and DNA was diluted to 10 ng/μL for PCR amplification. Prokaryotic universal primers 515F (5'-GTGYCAGCMGCCGCGGTA-3') and 909R (5'-CCCCGYCAATTCMTTTRAGT-3') (Tamaki et al., 2011) were used to amplify the V4-V5 hypervariable region of 16S rRNA genes in IM. A 12-nt tag sequence was attached to the 5' end of primer 515F for subsequent sample sorting. Library construction and Illumina HiSeq PE250 sequencing were completed by Guangdong Melikang Bio-Science Ltd., China (Foshan, Guangdong, China) (Li et al., 2022).

The raw reads were merged using FLASH 1.2.8 software and quality screened using QIIME 1.9.0 (Caporaso et al., 2010). Sequences that did not match the primers, sequences less than 300 bp in length, containing ambiguous base "N", and with an average base quality less than 30 were removed (Ni et al., 2017). Subsequently, chimeras were detected and removed using the UCHIME program (Edgar et al., 2013) to obtain high-quality sequences for further analysis. *De novo* classification of operational taxonomic units (OTUs) was performed using Usearch software (http://drive5.com/usearch/) at 97% similarity. QIIME 1.9.0 (Caporaso et al., 2022) was used to calculate α-diversity indices (i.e., OTU number, Shannon, Chao1, aand Simpon indices), and weighted and

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unweighted UniFrac distance matrices, and perform principal coordinate ranking analysis (PCoA). Taxonomy of each OUT was assigned using the RDP classifier (Wang et al., 2007) with Greengenes gg_13_8 database (http://qiime.org/home_static/dataFiles.html). Metabolic characteristics of IM were predicted using the PICRUSt software (Langille et al., 2013) based on the IM compositions.

Data analysis

Data are presented as mean ± standard deviation (S.D.). One-way ANOVA and Tukey-Kramer pairwise comparisons were performed using R 3.5.1 (R Core Team, 2013). Non-parametric multivariate analysis of variance (PERMANOVA) (Anderson et al., 2001) was performed using the R vegan package (Dixon, 2003). Krukal-Wallis H test was performed using STAMP software to screen for significantly different taxa (Parks et al., 2014). The R pheatmap package (R Core Team, 2013) was used to draw heatmap. The significantly differential taxa were also screened using the linear discriminant analysis effect size (LEfSe) (Segata et al., 2011). P-value < 0.05 was considered as statistical significance.

Results

Baicalin significantly reduced weight gain and insulin resistance induced by HFD

The body weight of HFD mice was significantly higher than that of NCD mice. After baicalin treatment, the body weight of HFD mice decreased significantly, although it was still significantly higher than that of NCDBCL mice (One-way ANOVA, $p < 0.05$; *Fig. 1A*). Tukey-Kramer post-hoc test results indicated that starting from the fourth week, the body weight of HFD mice significantly higher than those of NCD and NCDBCL mice. Although the body weight of HFDBCL mice was slightly lighter than that of HFD mice, the difference was not significant (*Appendix 1*). ITT results showed that the BGLs of HFD mice were significantly higher than those of NCD mice, and the BGLs were significantly decreased after baicalin treatment (One-way ANOVA, $p < 0.05$; *Fig. 1B*). Tukey-Kramer post-hoc test results indicated that the blood glucose levels of HFDBCL mice at different times were significantly lower than those of HFD mice, although the blood glucose levels of HFDBCL mice were still higher than those of NCD mice (*Appendix 2*). These results indicated that HFD significantly increased the body weight and insulin resistance of mice, and baicalin treatment could slightly reduce the increase in body weight and the enhancement of insulin resistance in HFD mice.

Baicalin reduced liver inflammation and liver fat accumulation in mice

Oil red staining of tissue sections showed that there was a large amount of fat accumulation in the liver of HFD mice, and the fat accumulation was significantly reduced after baicalin treatment (*Fig. 2*). F4/80 immunohistochemical results showed that the proportion of macrophages in the liver of HFD mice was significantly increased, and baicalin treatment significantly reduced the proportion of macrophages in the liver of HFD mice (One-way ANOVA, $p < 0.05$; *Fig. 3A-D* and *I*). CD4 immunofluorescence results showed that the relative proportion of T cells in the liver of HFD mice was significantly higher than that of NCD group, and the number of T cells in the liver of HFDBCL mice was significantly lower than that of HFD group (One-way ANOVA, $p < 0.05$; *Fig. 3E-I*). The results indicated that HFD significantly increased

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liver fat accumulation and the proportion of macrophages and T cells in the liver of mice. Baicalin treatment significantly reduced the fatty inflammation and fat accumulation in the liver of mice induced by HFD.

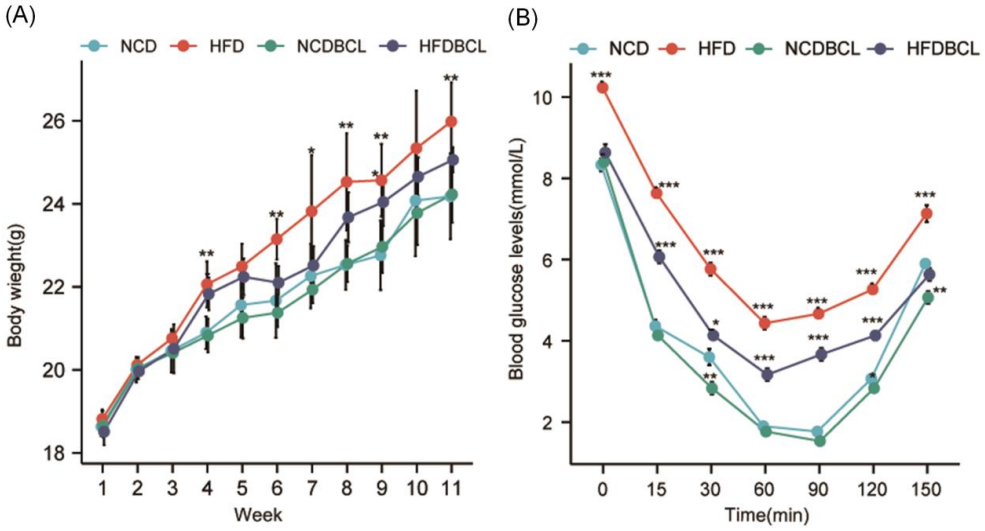

Figure 1. *Body weight (A) and insulin tolerance (B) of mice fed with different diets. NCD, HFD, NCDBCL, and HFDBCL indicate normal control, high-fat diet, baicalin-treated normal control and baicalin-treated high-fat diet groups, respectively. *p < 0.05; **p < 0.01; ***p < 0.001*

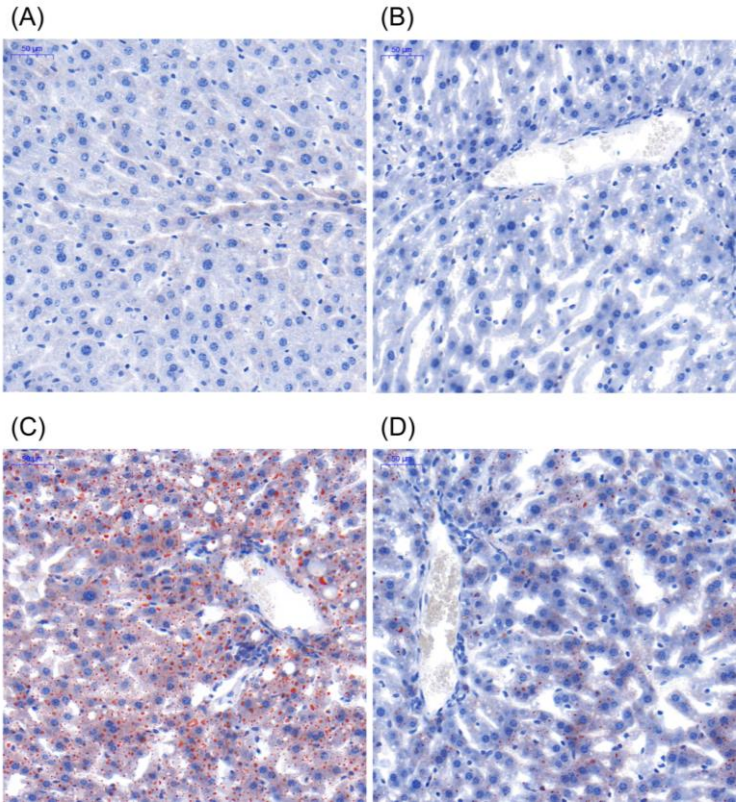

Figure 2. *Liver histological sections of mice with different treatments. Adipocytes in the sections were stained with oil red. (A), normal control group; (B), baicalin-treated normal control group; (C), high-fat diet group; (D), baicalin-treated high-fat diet group*

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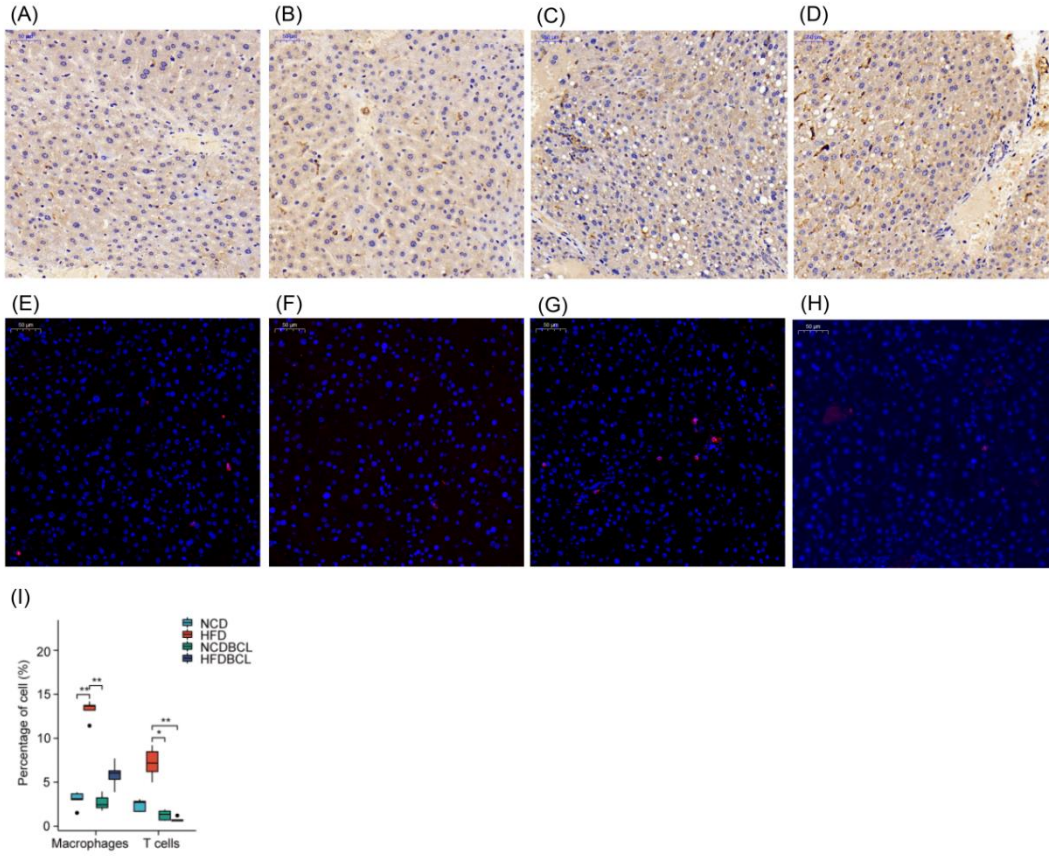

Figure 3. *Histological analysis of mice liver with different treatments. (A) - (D), F4/80 immunohistochemical analysis of the effect of berberine on the number of liver macrophages in mice with different treatments. Blue stain indicates the nucleus, whereas brown stain marks macrophages. (A), (B), (C), and (D) were normal control (NCD), high-fat diet (HFD), baicalin-treated NCD (NCDBCL), and baicalin-treated HFD (HFDBCL) groups, respectively. (E) - (H), CD4 immunofluorescence detection showing the effect of berberine on the number of T cells in the mice livers with different treatment. Blue stain indicates the nucleus, whereas red stain labels T cells. (E), (F), (G), and (H) were NCD, HFD, NCDBCL, and HFDBCL groups, respectively. (I) Statistical results of the number of macrophages and T cells. *$p < 0.05$; **$p < 0.01$*

Baicalin significantly changed the structure and metabolic characteristics of IM in HFD mice

To exclude the interference of different sequencing depths on the results, 98230 high-quality sequences were finally randomly selected from each sample for subsequent analysis. There was significant difference in Shannon index of IM between NCD and HFDBCL groups ($p < 0.05$), whereas there was no significant difference in the other α-diversity indices of IM among the groups ($p > 0.05$; *Table 1*).

Except for a few sequences that could not be determined at the phylum level, a total of 20 phyla were detected in mice fecal samples, among which Bacteroidetes, Firmicutes and Proteobacteria were the dominant phyla (relative abundance > 1% in at least one sample). In the NCD group, the proportions of Bacteroidetes, Firmicutes and Proteobacteria were $80.44\% \pm 0.91\%$, $17.84\% \pm 0.82\%$ and $1.29\% \pm 0.06\%$, respectively. In the HFD mice, Bacteroidetes, Firmicutes, and Proteobacteria accounted

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for 8.31% ± 0.54%, 89.16% ± 0.58%, and 1.08% ± 0.02%, respectively. In the NCDBCL mice, the proportions of Bacteroidetes, Firmicutes, and Proteobacteria were 64.21% ± 0.43%, 33.93% ± 0.42% and 1.39% ± 0.01%, respectively. In the HFDBCL mice, the proportions of Bacteroidetes, Firmicutes, and Proteobacteria were 7.79% ± 0.26%, 79.41% ± 0.21%, and 11.91% ± 0.33%, respectively (*Fig. 4A*). PCoA based on weighted UniFrac distance combined with PERMANOVA test showed significant differences in IM among the groups (PERMANOVA, $p < 0.05$; *Fig. 4B*).

Table 1. *α-diversity indices of mice intestinal microbiota with different treatments. NCD, HFD, NCDBCL, and HFDBCL indicate normal control, high-fat diet, baicalin-treated of normal control and baicalin-treated of high-fat diet groups, respectively. Different letters in the upper right corner of the number indicate significant differences ($p < 0.05$)*

Group	OTU number	Shannon index	Chao1 index	Simpson index
NCD	2343.33 ± 34.91	7.07 ± 0.02a	3302.45 ± 135.35	0.97 ± 0.00
HFD	2293.33 ± 91.51	7.61 ± 0.38ab	3381.11 ± 133.42	0.99 ± 0.00
NCDBCL	2439.00 ± 32.05	7.46 ± 0.01ab	3411.29 ± 169.24	0.97 ± 0.01
HFDBCL	2335.00 ± 34.39	7.65 ± 0.01b	3353.45 ± 40.79	0.99 ± 0.00

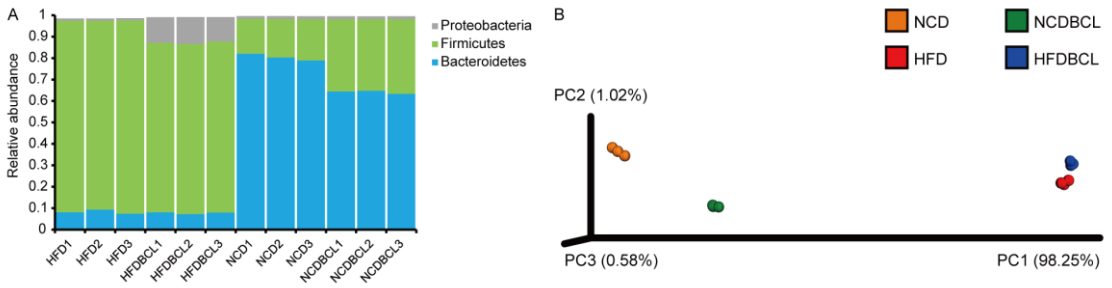

Figure 4. *Dominant phylum composition (A) and PCoA profile based on OTU composition (B) of mice with different treatments. NCD, HFD, NCDBCL, and HFDBCL indicate normal control, high-fat diet, baicalin-treated normal control, and baicalin-treated high-fat diet groups, respectively*

At the genus level, a total of 22 dominant genera were detected in the mice IM (relative abundance in at least one sample > 1%) (*Fig. 5A*), and the relative abundance of these genera varied between groups (*Fig. 5B*). *Prevotella* and unidentified genera in Bacteroidetes, Bacteroidia, S24_7, and Prevotellaceae were significantly enriched in the IM of NCD mice. *Ruminococcus*, *Clostridium*, *Odoribacter*, *Clostridia*, and unidentified genera in Firmicutes, Erysipelotrichi, Clostridiales, Erysipelotrichales, Lachnospiraceae, Erysipelotrichaceae, Odoribacteraceae and Rikenellaceae were significantly enriched in the IM of HFD mice. *Lactobacillus*, *Coprococcus*, *Bacteroides*, and unidentified genera in Lactobacillales, Bacilli, Bacteroidaceae and Lactobacillaceae were significantly enriched in the IM of NCDBCL mice. *Escherichia*, *Oscillospira*, *Roseburia*, *Klebsiella*, *Citrobacter*, and unidentified genera in Gammaproteobacteria, Proteobacteria, Enterobacteriales, Bacteroidales and Ruminococcaceae were significantly enriched in the IM of HFDBCL mice (*Fig. 5*). Moreover, although our results showed that the IM of HFDBCL mice was significantly

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different from that of HFD mice, this alteration was not restored to the level of NCD mice. Furthermore, the IM composition of HFDBCL mice more closely those of HFD mice but those of NCD mice (*Fig. 5*).

PICRUSt results showed that both HFD and baicalin treatment significantly changed the metabolic characteristics of IM compared with the NCD mice, and baicalin treatment also significantly changed the metabolic characteristics of IM in HFD mice (PERMANOVA, $p < 0.05$; *Fig. 6*). Baicalin treatment significantly reduced the relative abundance of genes involved in cysteine and methionine metabolism, glycerophosphatidylcholine metabolism in the IM metagenome of both NCD and HFD mice (One-way ANOVA, $p < 0.05$; *Fig. 6*). Compared with NCD mice, the relative abundance of genes involved in bisphenol degradation, glycolysis/gluconeogenesis, tetracycline biosynthesis, ansamicin biosynthesis, chloralkanes and chloralkanes degradation, pentose phosphate pathway, porphyrin and chlorophyll metabolism, starch and sucrose metabolism, glyceride metabolism, fatty acid biosynthesis, and linoleic acid metabolism in the IM metagenome of HFD mice were significantly enhanced. These genes were significantly decreased through baicalin treatment, although not to the level of NCD mice (One-way ANOVA, $p < 0.05$; *Fig. 6*). In addition, although compared with the NCD mice, the relative abundances of IM genes involved in steroid hormone biosynthesis, atrazine degradation, flavonoid and flavonol biosynthesis, flavonoid biosynthesis, D-arginine and D-ornithine metabolism, selenium compound metabolism, nitrotoluene degradation, protein kinase, benzoic acid degradation, pentose and glucuronic acid interconversion, fructose and mannose metabolism, xylene degradation, ketone body synthesis and degradation, dioxin degradation, retinol metabolism, chlorocyclohexane and chlorobenzene degradation, glyoxylate and dicarboxylic acid metabolism, α-linolenic acid metabolism, ascorbic acid and uronic acid metabolism, unsaturated fatty acid biosynthesis, caprolactam degradation, styrene degradation, butyric acid metabolism, and propionic acid metabolism were significantly increased in the HFD mice, baicalin treatment did not only significantly reduce the relative abundances of these genes, but also significantly increased the relative abundances of these genes (One-way ANOVA, $p < 0.05$; *Fig. 6*).

Discussion

Previous studies have shown that baicalin has a protective effect on hyperlipidemia stress and redox imbalance induced by HFD in rats (Shambhoo et al., 2020), and the reason may be that baicalin accelerates lipid influx into mitochondria for oxidation by activating carnitine palmitoyltransferase 1 (CPT1), the controlling enzyme of fatty acid oxidation in the liver (Dai et al., 2018). Experiments on fatty HepG2 cell model treated with high oleic acid also showed that baicalin could reduce the levels of triglyceride and total cholesterol in fatty liver HepG2 cells (Sun et al., 2020). Our results showed that HFD could significantly increase the body weight and liver fat content of mice, and baicalin treatment could significantly improve the HFD-induced body weight gain and liver fat cell increase, suggesting that baicalin could effectively regulate liver lipid metabolism. Other studies have shown that HFD causes a significant increase in fasting blood glucose concentration and insulin resistance in mice (Fu et al., 2014), and our study also exhibited similar results, and the increase trends were significantly improved after baicalin treatment. The reason may be that baicalin promotes the transduction of P38MAPK/PGC-1α/GLUT4 and AKT/AS160/GLUT4 signaling pathways by activating

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GALR2, increasing GLUT4 expression and membrane translocation in muscle cells, thereby reducing insulin resistance (Fang et al., 2017). Whether this process is related to the metabolism of IM needs further experimental analysis.

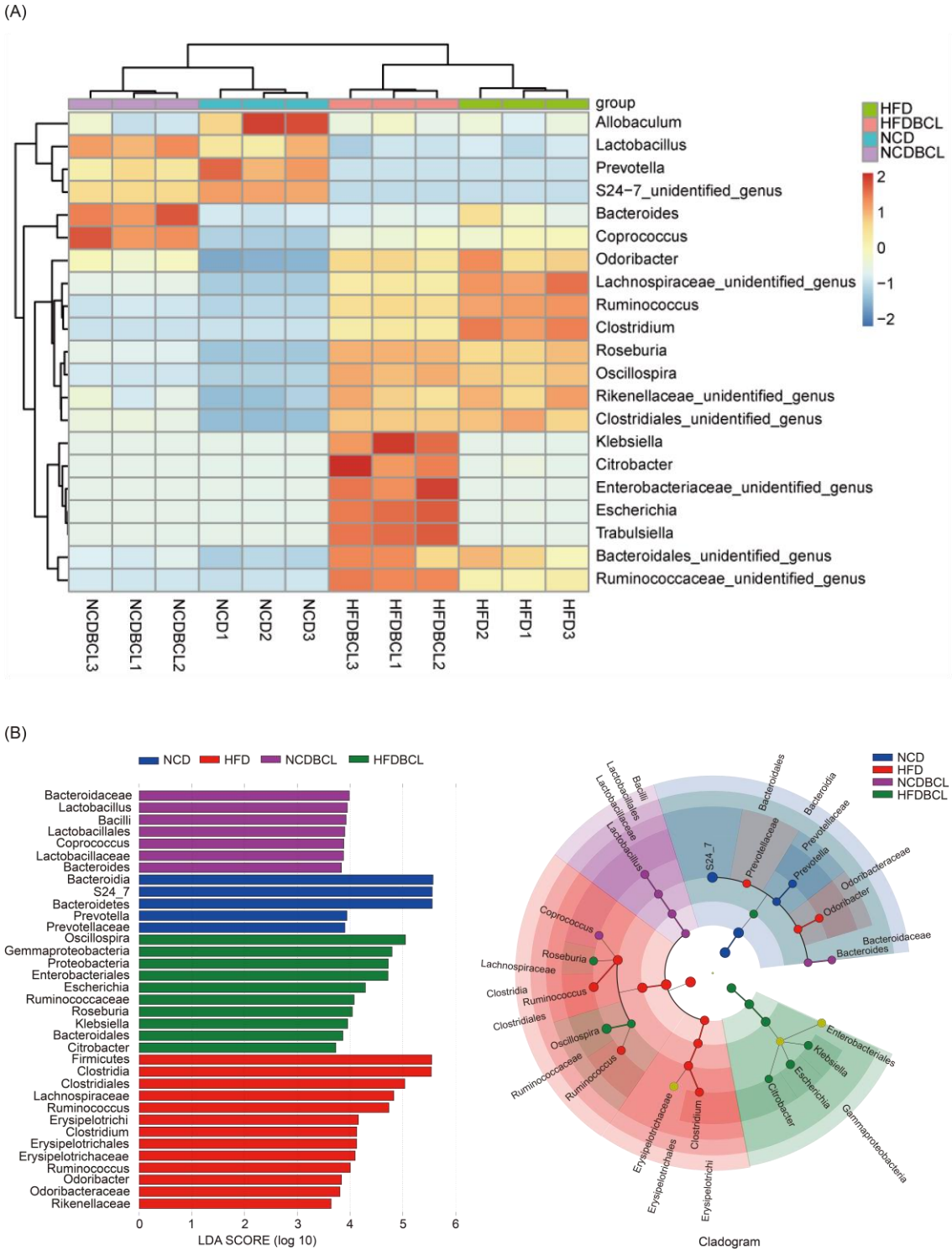

Figure 5. *Heatmap profile (A) and LEfSe result (B) of dominant genera in mice intestinal microbiota with different treatments. NCD, HFD, NCDBCL, and HFDBCL indicate normal control, high-fat diet, baicalin-treated normal control and baicalin-treated high-fat diet groups, respectively*

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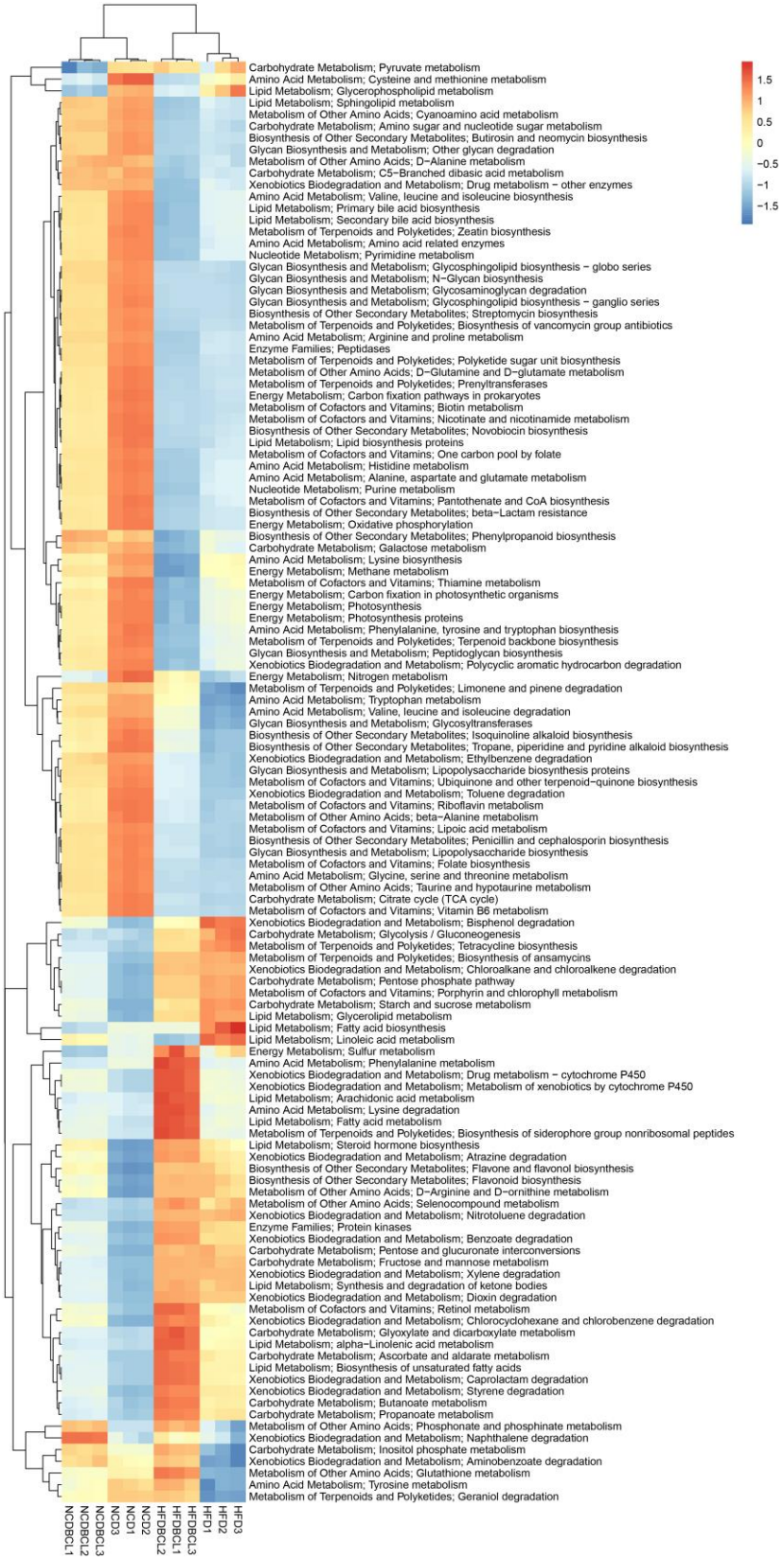

Figure 6. *Heatmap profile of intestinal microbial genes of mice with different treatments. NCD, HFD, NCDBCL, and HFDBCL indicate normal control, high-fat diet, baicalin-treated normal control, and baicalin-treated high-fat diet groups, respectively*

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Macrophages and T cells play important role in the phagocytosis, cellular immunity, and molecular immunology (Lee et al., 2022; Ma et al., 2023), and they are closely related to the occurrence of fatty metabolic diseases in the liver (Wiering and Tacke, 2022). Excessive energy intake and fat accumulation can activate cells and related cytokines mainly involved in innate immunity (Cani et al., 2007; Cai et al., 2005). Meanwhile, various mature immune cells (including mast cells, macrophages, and dendritic cells) and adipocytes are further recruited and activated to jointly enhance the inflammatory response (Lumeng et al., 2011). In the present study, the HFD increased the percentages of macrophages and T cells in the liver of mice, and both percentages were significantly decreased after baicalin treatment (*Fig. 3*). These results indicated that HFD could hinder fat metabolism and increase inflammatory response in the liver of mice, and baicalin treatment could significantly relieve this situation.

There are many microorganisms in the intestinal tract of vertebrates, among which the number of bacteria is equivalent to that of their own cells (Ron et al., 2016), and they are closely related to the nutrition, metabolism, and immunity of the host (Jin et al., 2020). Among these microorganisms, Bacteroidetes, Firmicutes and Proteobacteria are the most important members (Yang et al., 2017). In this study, the analysis of the dominant phyla in the mouse gut also showed that, except for a few sequences that could not be determined at the phylum level, a total of 20 phyla were detected in mouse fecal samples, of which Bacteroidetes, Firmicutes, and Proteobacteria were the dominant phyla (*Fig. 4A*). Previous studies showed that OTUs number in HFD group was significantly higher than that in NCD group, and it was significantly decreased after baicalin treatment with high dose (50 $mg \cdot kg^{-1} \cdot d^{-1}$) (Liu et al., 2016). The Shannon index of the HFD group was significantly lower than that of the NCD group, while the low-dose baicalin group (25 $mg \cdot kg^{-1} \cdot d^{-1}$) was significantly higher than that of the NCD group. The Chao1 index of the HFD group was higher than that of the NCD group, while there was no significant difference after baicalin treatment (Liu et al., 2016). Our results indicated that there were significant differences in Shannon index of IM between NCD and HFDBCL groups ($p < 0.05$), whereas there was no significant difference in other α-diversity of IM among the groups. It is speculated that this may be caused by the physiological condition of the experimental mice, the intervention concentration of baicalin and the feeding conditions of the mice.

The ratio of Firmicutes to Bacteroidetes not only affects carbohydrate metabolism and the production of short-chain fatty acids, but also leads to insulin resistance and increased ghrelin production. The proportion of Bacteroidetes increased relative to that of Firmicutes when obese individuals dieted to lose weight. In contrast, when obese individuals return to their previous diet and gain weight, the proportion of Firmicutes increases (Anthony et al., 2017). Similar results were found in our results. In the HFD group, the proportions of Firmicutes and Bacteroidetes were $89.16\% \pm 0.58\%$ and $8.31\% \pm 0.54\%$, respectively, while in the NCD group, the proportion of Firmicutes and Bacteroidetes were $17.84\% \pm 0.82\%$ and $80.44\% \pm 0.91\%$, respectively. Moreover, Firmicutes and unidentified genera in Blastomyces, Erysipelothrix and Erysipelothrix of Firmicutes were significantly enriched in fecal samples from HFD mice. After baicalin treatment, an unidentified genus of Bacteroidetes was significantly enriched in the IM of HFD mice, and unidentified genera of Bacteroidetes and Bacteroidetes were significantly enriched in the IM of NCD mice. *Prevotella* exists in the human microbiome, and its species diversity and function are affected by factors such as host's diet, lifestyle, age, gender, and geographical location (Adrian et al., 2021). We detected

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an unidentified genus of Prevotaceae and *Prevotella* were significant enriched in the IM of NCD mice. *Lactobacillus* is negatively correlated with the severity of autoimmune encephalomyelitis, whereas Rikenellaceae and *Clostridium* are positively correlated with it (He et al., 2019). Consistent with this finding, unidentified genera in Clostridiales and Rikinaceae was significantly enriched in the IM of HFD mice. *Lactobacillus* was significantly enriched in the IM of NCDBCL mice. Crohn's disease was associated with an increase in flagellin-specific CD4 + memory T cells in a variety of Tricspirillaceae (Alexander et al., 2021), and we also detected significant enrichment of an unidentified genus in Tricspirillaceae and *Ruminococcus* in samples of HFD mice. *Oscillosporia* and *Citrobacter* negatively correlated with host inflammatory index (Konikoff et al., 2016; An et al., 2021). *Roseburia inulivorans* is enriched in the gut of healthy people and promotes short-chain fatty acid biosynthesis and secondary bile acid levels (Paramsothy et al., 2019). Our results show that in the HFDBCL mice, abundance of *Oscillosporia*, *Citrobacter*, and *Roseburia* were significantly enhanced (Paramsothy et al., 2019). *Ruminococcus gnavus* plays an up-regulated role in the expression of intestinal oxidative stress-related genes in patients with inflammatory bowel disease (Hall et al., 2017). Our results showed that this bacterium was significantly enriched in the IM of HFD mice, but not detected in the IM of HFDBCL mice. These results suggest that HFD induced chronic inflammatory response, and baicalin can be used to treat obesity-induced liver fat accumulation. During the treatment process, regulating IM structure is one of the important ways to improve lipid metabolism and inflammation.

A study on the IM and blood succinic acid concentration in obesity patients showed that obesity was related to increased blood succinic acid and abnormal glucose metabolism. Prevotellaceae produces succinic acid, Odoribacteraceae and Clostridiaceae degraded succinic acid, and the ratio of succinic acid producing/degrading bacteria significantly affects the concentration of blood succinic acid (Serena et al., 2018). However, in our study, we found that unidentified genera in Clostridiales and Odoribacteraceae, and *Clostridium* were significantly enriched in the IM of HFD mice. Moreover, patients with steatosis have an increase in endotoxin-producing bacteria (especially Proteobacteria) (Hoyles et al., 2018), and HFD alters the biological function of mitochondria in the colonic epithelium, thereby increasing choline degradation to trimethylamine in *Escherichia coli* (Yoo et al., 2021). Periodontitis led to an increase in a variety of *Klebsiella* and Enterobacter in oral cavity (Kitamoto et al., 2020), whereas our results showed that unidentified genera in γ-Proteobacteria, Enterobacteriales and Ruminococcaceae, as well as *Escherichia* and *Klebsiella* were significantly enriched in the IM of HFDBCL mice. The specific reasons and related mechanisms need to be further studied.

Baicalin effectively reduced lipid accumulation in the liver of the HFD mice, but it had no significant effect on body weight, blood glucose, and liver fat accumulation in the NCD mice (*Figs. 1* and *2*). Previous studies of patients with steatosis exhibited a significantly negative correlation between steatosis and *Coprococcus* (Alferink et al., 2020). When diet-induced obesity status is improved, the abundance of *Bacillus* reduced (Ziętak et al., 2016). *Bacteroides* regulate hepatic lipid metabolism and reduce NAFLD through activating the *Bacteroides* -folate-liver axis (Qiao et al., 2020). It is worth mentioned that *Coprococcus*, *Bacillus* and *Bacteroides* were significantly enriched in the IM of HFDBCL mice, suggesting that baicalin may also have a beneficial effect on the health of HFD mice.

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Sex differences in liver metabolic activity have been reported extensively (Maggi, 2022). The liver of males is good in alcohol clearance and lipid metabolism, while the liver of females is better in cholesterol metabolism (Ullah et al., 2021). These metabolic differences lead to sex differences in the incidence rates of liver diseases and complications. For instance, approximately two-thirds of all liver-related deaths occur in males (Devarbhavi et al., 2023). A greater prevalence of NAFLD in men than in women (39.7% [36.6-42.8] vs 25.6% [22.3-28.8]; $p < 0.001$) (Browning et al., 2004; Riazi et al., 2022). Moreover, men have more cardiovascular complications associated with obesity than women (Palmisano et al., 2018). Therefore, we analyzed the impact of baicalin on male mice. This does not mean that the results in female mice are not important, and further research is needed on the impact of baicalin on female mice.

Conclusions

HFD induced liver inflammation and excessive fat accumulation in mice, and baicalin alleviated these effects, which might be related to the regulation of baicalin on the structure of IM. This study helps to improve our understanding of host-IM interactions in the treatment of obesity with baicalin, and provides a theoretical basis for further studies on the mechanism of IM in the treatment of metabolic syndromes with baicalin.

REFERENCES

[1] Adrian, T., Edoardo, P., Giulia, M., Danilo, E., Nicola, S. (2021): Prevotella diversity, niches and interactions with the human host. – Nature Reviews Microbiology 19(9): 585-599.

[2] Alexander, K. L., Zhao, Q., Reif, M., Rosenberg, A. F., Mannon, P. J., Duck, L. W., Elson, C. O. (2021): Human microbiota flagellins drive adaptive immune responses in Crohn's disease. – Gastroenterology 161(2): 522-535.

[3] Alferink, L. J. M., Radjabzadeh, D., Erler, N. S., Vojinovic, D., Medina-Gomez, C., Uitterlinden, A. G., Knegt, R. J. D., Amin, N., Ikram, M. A., Janssen, H. L. A., Jong, J. C. K., Metselaar, H. J., Duijn, C. M., Kraaij, R., Murad, S. D. (2020): Microbiomics, metabolomics, predicted metagenomics and hepatic steatosis in a population-based study of 1355 adults. – Hepatology 73(3): 968-982.

[4] An, J. Q., Zhao, X., Wang, Y. L., Noriega, J., Noriega, J., Gewirtz, A. T., Zou, J. (2021): Western-style diet impedes colonization and clearance of *Citrobacter rodentium*. – PLoS Pathogens 17(4): e1009497.

[5] Anderson, M. J. (2001): A new method for non-parametric multivariate analysis of variance. – Austral Ecology 26: 32-46.

[6] Anthony, L. K. (2017): The microbiome and risk for obesity and diabetes. – Journal of the American Medical Association 317(4): 355-356.

[7] Browning, J. D., Szczepaniak, L. S., Dobbins, R., Nuremberg, P., Horton, J. D., Cohen, J. C., Grundy, S. M., Hobbs, H. H. (2004): Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. – Hepatology 40(6): 1387-1395.

[8] Bui, T. I., Britt, E. A., Muthukrishnan, G., Gill, S. F. (2023): Probiotic induced synthesis of microbiota polyamine as a nutraceutical for metabolic syndrome and obesity-related type 2 diabetes. – Frontiers in Endodcrinology 13: 1094258.

[9] Cai, D., Yuan, M., Frantz, D. F., Melendez, P. A., Hansen, L., Lee, J., Shoelson, S. E. (2005): Local and systemic insulin resistance resulting from hepatic activation of IKK-beta and NF-kappaB. – Nature Medicine 11(2): 183-90.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2106_59415959

[10] Cani, P. D., Amar, J., Iglesias, M. A., Poggi, M., Knauf, C., Bastelica, D., Neyrinck, A. M., Fava, F., Tuohy, K. M., Chabo, C., Waget, A., Delmée, E., Cousin, B., Sulpice, T., Chamontin, B., Ferrières, J., Tanti, J. F., Gibson, G. R., Casteilla, L., Delzenne, N. M., Alessi, M. C., Burcelin, R. (2007): Metabolic endotoxemia initiates obesity and insulin resistance. – Diabetes 56(7): 1761-1772.

[11] Caporaso, J. G., Kuczynski, J., stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., Fierer, N., Peña, A. G., Goodrich, J. K., Gordon, J. I., Huttley, G. A., Kelley, S. T., Knights, D., Koenig, J. E., Ley, R. E., Lozupone, C. A., McDonald, D., Muegge, B. D., Pirrung, M., Reeder, J., Sevinsky, J. R., Turnbaugh, P. J., Walters, W. A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R. (2010): QIIME allows analysis of high-throughput community sequencing data. – Nature Methods 7(5): 335-336.

[12] Dai, J. Y., Liang, K., Zhao, S., Jia, W. T., Liu, Y., Wu, H. K., Lv, J., Chen, C., Chen, T., Zhuang, S. T., Hou, X. M., Zhou, S. J., Zhang, X. N., Chen, X. W., Huang, Y. Y., Xiao, R. P., Wang, Y. L., Luo, T. P., Xiao, J. Y., Wang, C. (2018): Chemoproteomics reveals baicalin activates hepatic CPT1 to ameliorate diet-induced obesity and hepatic steatosis. – Proceedings of the National Academy of Sciences of the United States of America 115(26): E5896-E5905.

[13] DeMarco, V. G., Aroor, A. R., Sowers, J. R. (2014): The pathophysiology of hypertension in patients with obesity. – Nature Reviews Endocrinology 10: 364-376.

[14] Devarbhavi, H., Asrani, S. K., Arab, J. P., Nartey, Y. A., Pose, E., Kamath, P. S. (2023): Global burden of liver disease: 2023 update. – Journal of Hepatology 79: 516-537.

[15] Dinda, B., Dinda, S., DasSharma, S., Banik, R., Chakraborty, A., Dinda, M. (2017): Therapeutic potentials of baicalin and its aglycone, baicalein against inflammatory disorders. – European Journal of Medicinal Chemistry 131: 68-80.

[16] Dixon, P. (2003): VEGAN, a package of R functions for community ecology. – Journal of Vegetation Science 14: 927-930.

[17] Edgar, R. C. (2013): UPARsE: highly accurate OTU sequences from microbial amplicon reads. – Nature Methods 10: 996-998.

[18] Fang, P. H. (2017): Regulative Mechanism and Effects of Baicalin on Insulin Resistance through GALR2/GLUT4 Pathway. – Yangzhou University, Yangzhou.

[19] Fu, Y., Luo, J., Jia, Z. Q., Zhen, W., Zhou, K. Q., Gilbert, E., Liu, D. M. (2014): Baicalein protects against type 2 diabetes via promoting islet β-cell function in obese diabetic mice. – International Journal of Endocrinology 2014: 846742.

[20] Gao, W., Xu, B., Zhang, Y., Liu, S., Duan, Z., Chen, Y., Zhang, X. (2022): Baicalin attenuates oxidative stress in a tissue-engineering liver model of NAFLD by scavenging reactive oxygen species. – Nutrients 14(3): 541.

[21] Hall, A. B., Yassour, M., Sauk, J., Garner, A., Jiang, X. F., Arthur, T., Lagoudas GK, Vatanen T., Fornelos N., Wilson R., Bertha M., Cohen M., Garber J., Khalili H. M. Gevers D., Ananthakrishnan A. N., Kugathasan S., Lander E. S., Blainey P., Vlamakis H. M. Xavier R. J., Huttenhower, C. (2017): A novel *Ruminococcus gnavus* clade enriched in inflammatory bowel disease patients-lecture. – Genome Medicine 9(1): 103.

[22] He, B. K., Hoang, T. K., Tian, X. J., Taylor, C. M., Blanchard, E., Luo, M., Bhattacharjee, M. B., Freeborn, J., Park, S. Y., Couturier, J., Lindsey, J. W., Tran, D. Q., Rhoads, J. M., Liu, Y. Y. (2019): *Lactobacillus reuteri* reduces the severity of experimental autoimmune encephalomyelitis in mice by modulating gut microbiota. – Frontiers in Immunology 10: 385.

[23] Hoyles, L., Fernández-Real, J. M., Federici, M., Serino, M., Abbott, J., Charpentier, J., Heymes, C., Luque, J. L., Anthony, E., Barton, R. H., Chilloux, J., Myridakis, A., Martinez-Gili, L., Moreno-Navarrete, J. M., Benhamed, F. M., Azalbert, V., Blasco-Baque, V., Puig, J., Xifra, G., Ricart, W., Tomlinson, C., Woodbridge, M., Cardellini, M., Davato, F., Cardolini, I., Porzio, O., Gentileschi, P., Lopez, F., Foufelle, F., Butcher, S. A., Holmes, E., Nicholson, J. K., Postic, C., Burcelin, R., Dumas, M. E. (2018):

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2106_59415959

Molecular phenomics and metagenomics of hepatic steatosis in non-diabetic obese women. – Nature Medicine 24(7): 1070-1080.

[24] Jin, J. P., Jia, J. L., Zhang, L. P., Chen, Q., Zhang, X. Y., Sun, W. B., Ma, C. M., Xu, F. F., Zhan, S. J., Ma, L. M., Zhou, G. H., Chen, Q. X. (2020): Jejunal inflammatory cytokines, barrier proteins and microbiome-metabolome responses to early supplementary feeding of Bamei suckling piglets. – BMC Microbiology 20(1): 169.

[25] Kitamoto, S., Nagao-Kitamoto, H., Jiao, Y. Z., Gillilland, M. G., Hayashi, A., Imai, J., Sugihara, K., Miyoshi, M., Brazil, J. C., Kuffa, P., Hill, B. D., Rizvi, S. M., Wen, F., Bishu, S., Inohara, N., Eaton, K. A., Nusrat, A., Lei, Y. L., Giannobile, W. V., Kamada, N. (2020): The intermucosal connection between the mouth and gut in commensal pathobiont-driven colitis. – Cell 182(2): 447-462.

[26] Klaauw, A. A., Farooqi, I. S. (2015): The hunger genes: pathways to obesity. – Cell 161: 119-132.

[27] Konikoff, T., Gophna, U. (2016): Oscillospira: a central, enigmatic component of the human gut microbiota. – Trends in Microbiology 24(7): 523-524.

[28] Langille, M. G. I., Zaneveld, J., Caporaso, J. G., McDonald, D., Knights, D., Reyes, J. A., Clemente, J. C., Burkepile, D. E., Thurber, R. L. V., Knight, R., Beiko, R. G., Huttenhower, C. (2013): Gene sequences. – Nature Biotechnology 31(9): 814-821.

[29] Lee, J. C., Green, M. D., Huppert, L. A., Chow, C., Pierce, R. H., Daud, A. I. (2022): The liver-immunity nexus and cancer immunotherapy. – Clinical Cancer Research 28(1): 5-12.

[30] Li, J. J., Li, J. L., Ni, J. J., Zhang, C. B., Jia, J. L., Wu, G. Y., Sun, H. Z., Wang, S. Z. (2022): Berberine relieves metabolic syndrome in mice by inhibiting liver inflammation caused by a high-fat diet and potential association with gut microbiota. – Frontiers in Microbiology 12: 752512.

[31] Lin, J. Y., Cai, Q. Y., Liang, B., Wu, L. Z., Zhuang, Y., He, Y. F., Lin, W. R. (2019): Berberine, a traditional Chinese medicine, reduces inflammation in adipose tissue, polarizes M2 macrophages, and increases energy expenditure in mice fed a high-fat diet. – Medical Science Monitor 25: 87-97.

[32] Liu, C. X. (2018): Gut microbiota: the effects of health, illness and medicines. – Chinese Journal of Antibiotics 43(1): 1-14.

[33] Liu, S. Y., Kuang, Z. Y., Zhang, R., Xie, W. Q., Chen, J. (2016): Regulatory effect of baicalin on metaflammation and intestinal flora. – Journal of Guangzhou University of Traditional Chinese Medcine 33(3): 372-376.

[34] Lumeng, C. N., Saltiel, A. R. (2011): Inflammatory links between obesity and metabolic disease. – Journal of Clinical Investigation 121(6): 2111-2117.

[35] Ma, S. B., Sun, B. F., Duan, S. Q., Han, J. J., Barr, T., Zhang, J. Y., Bissonnette, M. B., Kortylewski, M., He, C., Chen, J. J., Caligiuri, M. A., Yu, J. H. (2023): YTHDF2 orchestrates tumor-associated macrophage reprogramming and controls antitumor immunity through CD8+ T cells. – Nature Immunology 24(2): 255-266.

[36] Maggi, A. (2022): Sex and liver disease: the necessity of an overarching theory to explain the effect of sex on nonreproductive functions. – Endocrinology 163(1): bqab229.

[37] Ni, J., Li, X., He, Z., Xu, M. (2017): A novel method to determine the minimum number of sequences required for reliable microbial community analysis. – Journal of Microbiological Methods 139: 196-201.

[38] Palmisano, B. T., Zhu, L., Eckel, R. H., Stafford, J. M. (2018): Sex differences in lipid and lipoprotein metabolism. – Molecular Metabolism 15: 45-55.

[39] Paramsothy, S., Nielsen, S., Kamm, M. A., Deshpande, N. P., Faith, J. J., Clemente, J. C., Paramsothy, R., Walsh, A. J., Bogaerde, J. V. D., Samuel, D., Leong, R. W. L., Connor, S., Ng, W., Lin, E., Borody, T. J., Wilkin, M. R., Colomber, J. F., Mitchell, H. M., Kaakoush, N. O. (2019): Specific bacteria and metabolites associated with response to fecal microbiota transplantation in patients with ulcerative colitis. – Gastroenterology 156(5): 1440-1454.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2106_59415959

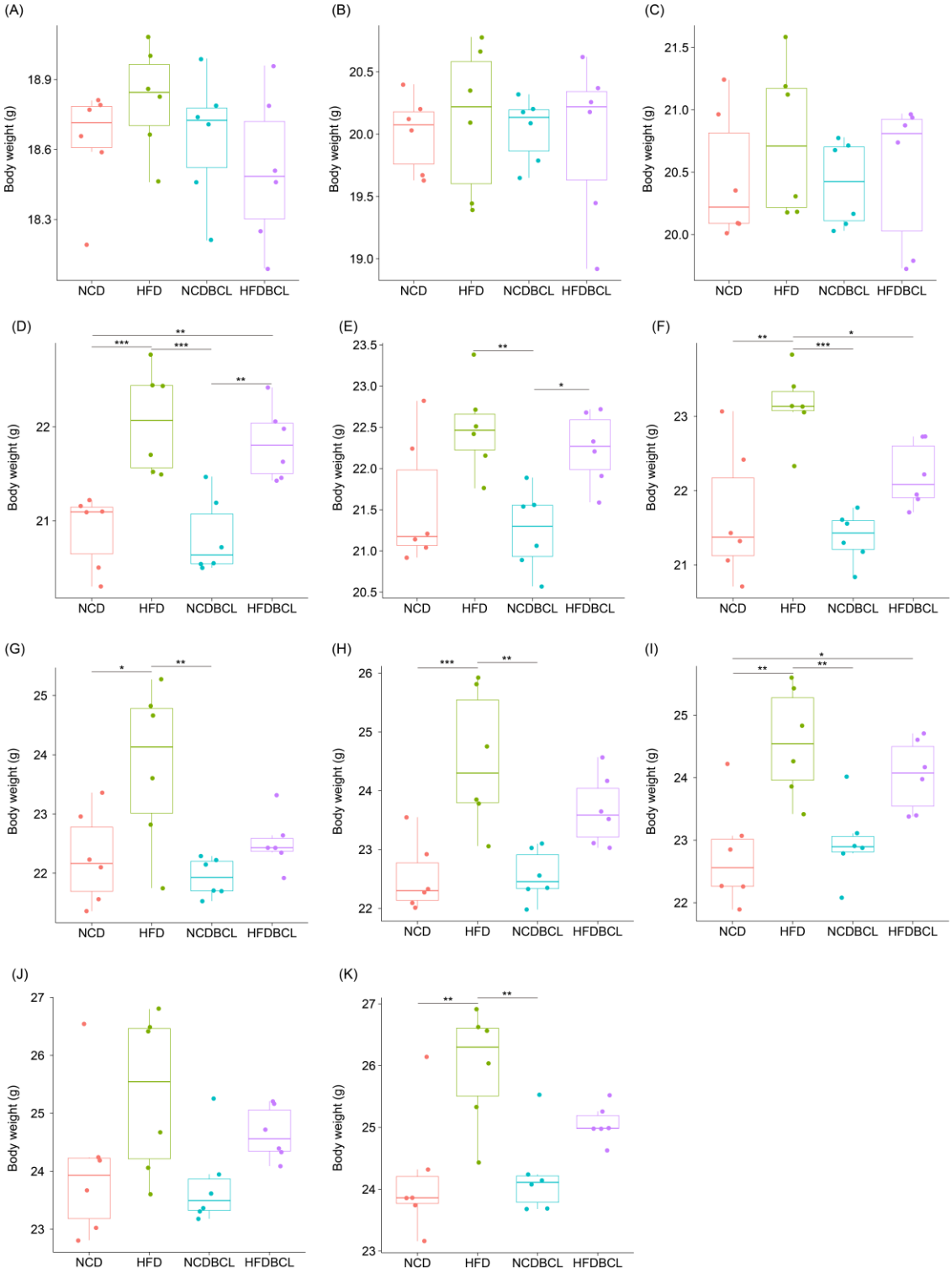
[40] Parks, D. H., Tyson, G. W., Hugenholtz, P., Beiko, R. G. (2014): STAMP: Statistical analysis of taxonomic and functional profiles. – Bioinformatics 30: 3123-3124.
[41] Polyzos, S. A., Goulis, D. G., Giouleme, O., Germanidis, G. S., Goulas, A. (2022): Anti-obesity medications for the management of nonalcoholic fatty liver disease. – Current Obesity Reports 11(3): 166-179.
[42] Qiao, S. S., Bao, L., Wang, K., Sun, S. S., Liao, M. F., Liu, C., Zhou, N., Ma, K., Zhang, Y. W., Chen, Y. H., Liu, S. J., Liu, H. W. (2020): Activation of a specific gut Bacteroides-folate-liver axis benefits for the alleviation of nonalcoholic hepatic steatosis. – Cell Reports 32(6): 108005.
[43] Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K. S., Manichanh, C., Nielsen, T., Pons, N., Levenez, F., Yamada, T., Mende, D. R., Li, J. H., Xu, J. M., Li, S. C., Li, D. F., Cao, J. J., Wang, B., Liang, H. Q., Zheng, H. S., Xie, Y. L., Tap, J., Lepage, P., Bertalan, M., Batto, J. M., Hansen, T., Paslier, D. L., Linneberg, A., Nielsen, H. B., Pelletier, E., Renault, P., Sicheritz-Ponten, T., Turner, K., Zhu, H. M., Yu, C., Li, S. T., Jian, M., Zhou, Y., Li, Y. R., Zhang, X. Q., Li, S. G., Qin, N., Yang, H. M., Wang, J., Brunak, S., Doré, J., Guarner, F., Kristiansen, K., Pedersen, O., Parkhill, J., Weissenbach, J., Consortium, M., Bork, P., Ehrlich, S. D., Wang, J. (2010): A human gut microbial gene catalogue established by metagenomic sequencing. – Nature 464(7285): 59-65.
[44] R Core Team (2013): R: A Language and Environment for Statistical Computing. – R Foundation for Statistical Computing. Vienna, Austria, URL http://www.R-project.org/.
[45] Radu, F., Potcovaru, C. G., Salmen, T., Filip, P. V., Pop, C., Carmen, F. B. (2023): The link between NAFLD and metabolic syndrome. – Diagnostics 13(4): 614.
[46] Riazi, K., Azhari, H., Charette, J. H., Underwood, F. E., King, J. A., Afshar, E. E., Swain, M. G., Congly, S. E., Kaplan, G. G., Shaheen, A. A. (2022): The prevalence and incidence of NAFLD worldwide: a systematic review and meta-analysis. – The Lancet Gastroenterology & Hepatology 7(9): 851-861.
[47] Ron, S., Shai, F., Ron, M. (2016): Are we really vastly outnumbered? Revisiting the ratio of bacterial to host cells in humans. – Cell 164(3): 337-340.
[48] Saltiel, A. R., Kahn, C. R. (2001): Insulin signalling and the regulation of glucose and lipid metabolism. – Nature 414: 799-806.
[49] Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W. S., Huttenhower, C. (2011): Metagenomic biomarker discovery and explanation. – Genome Biology 12: R60.
[50] Serena, C., Ceperuelo-Mallafré, V., Keiran, N., Queipo-Ortuño, M. I., Bernal, R., Gomez-Huelgas, R., Urpi-Sarda, M., Sabater, M., Pérez-Brocal, V., Andrés-Lacueva, C., Moya, A., Tinahones, F. J., Fernández-Real, M., Vendrell, J., Fernández-Veledo, S. (2018): Elevated circulating levels of succinate in human obesity are linked to specific gut microbiota. – ISME Journal 12(7): 1642-1657.
[51] Seth, P., Hsieh, P. N., Jamal, S., Wang, L., Stamler, J. S. (2019): Regulation of microRNA machinery and development by interspecies S-nitrosylation. – Cell 176(5): 1014-1125.
[52] Shah, P. A., Patil, R., Harrison, S. A. (2023): NAFLD-related hepatocellular carcinoma: the growing challenge. – Hepatology 77(1): 323-338.
[53] Shambhoo, S. T., Raushan, K., Akalabya, B., Syed, I. R. (2020): Baicalein maintains redox balance in experimental hyperlipidemic rats. – Archives of Physiology and Biochemistry 12: 1-9.
[54] Sun, W. L., Liu, P. P., Wang, T. Q., Wang, X. D., Zheng, W. L., Li, J. D. (2020): Baicalein reduces hepatic fat accumulation by activating AMPK in oleic acid-induced HepG2 cells and high-fat diet-induced non-insulin-resistant mice. – Food and Function 11(1): 711-721.
[55] Tamaki, H., Wright, C. L., Li, X., Lin, Q., Hwang, C., Wang, S., Thimmapuran, J., Kamagata, Y., Liu, W. T. (2011): Analysis of 16s rRNA amplicon sequencing options on the Roche/454 next-generation titanium sequencing platform. – PLoS ONE 6: e25263.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2106_59415959

[56] Tanti, J. F., Ceppo, F., Jager, J., Berthou, F. (2013): Implication of inflammatory signaling pathways in obesity-induced insulin resistance. – Frontiers in Endocrinology (Lausanne) 3: 181.
[57] Ullah, I., Shin, Y., Kim, Y., Oh, K. B., Hwang, S., Kim, Y.-I., Lee, J. W., Hur, T.-Y., Lee, S., Ock, S. A. (2021): Effect of sex-specific differences on function of induced hepatocyte-like cells generated from male and female mouse embryonic fibroblasts. – Stem Cell Research & Therapy 12: 79.
[58] Velagapudi, V. R., Hezaveh, R., Reigstad, C. S., Gopalacharyulu, P., Yetukure, L., Islam, S., Felin, J., Perkins, R., Borén, J., Oresic, M., Bäckhed, F. (2010): The gut microbiota modulates host energy and lipid metabolism in mice. – Journal of Lipid Research 51(5): 1101-1112.
[59] Wang, Q., Garrity, G. M., Tiedje, J. M., Cole, J. R. (2007): Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. – Applied and Environmental Microbiology 73: 5261-5267.
[60] Wiering, L., and Tacke, F. (2022): Treating inflammation to combat non-alcoholic fatty liver disease. – Journal of Endocrinology 256(1): e220194.
[61] Wu, Z., Chen, C., Miao, Y., Liu, Y., Zhang, Q., Li, R., Ding, L., Ishfaq, M., Li, J. (2019): Baicalin attenuates mycoplasma gallisepticum-induced inflammation via inhibition of the TLR2-NF-κB pathway in chicken and DF-1 cells. – Infection and Drug Resistance 12: 3911-3923.
[62] Yan, W. J., Ma, X. C., Gao, X. Y., Xue, X. H., Zhang, S. Q. (2016): Latest research progress in the correlation between baicalein and breast cancer invasion and metastasis. – Molecular and Clinical Oncology 4: 472-476.
[63] Yang, Y., Chen, G., Yang, Q., Ye, J., Cai, X. T., Tsering, P., Cheng, X. L., Hu, C. P., Zhang, S. Q., Cao, P. (2017): Gut microbiota drives the attenuation of dextran sulphate sodium-induced colitis by Huangqin decoction. – Oncotarget 8(30): 48863-48874.
[64] Yoo, W., Zieba, J. K., Foegeding, N. J., Torres, T. P., Shelton, C. D., Shealy, N. G., Byndloss, A. J., Cevallos, S. A., Gertz, E., Tiffany, C. R., Thomas, J. D., Litvak, Y., Nguyen, H., Olsan, E. E., Bennett, B. J., Rathmell, J. C., Major, A. S., Bäumler, A. J. M., Byndloss, M. X. (2021): High-fat diet-induced colonocyte dysfunction escalates microbiota-derived trimethylamine N-oxide. – Science 373(6556): 813-818.
[65] Zha, A., Cui, Z., Qi, M., Liao, S., Yin, J., Tan, B., Liao, P. (2020): Baicalin-copper complex modulates gut microbiota, inflammatory responses, and hormone secretion in DON-challenged piglets. – Animals 10: 1535.
[66] Ziętak, M., Kovatcheva-Datchary, P., Markiewicz, L. H., Ståhlman, M., Kozak, L. P., Bäckhed, F. (2016): Altered microbiota contributes to reduced diet-induced obesity upon cold exposure. – Cell Metabolism 23(6): 1216-1223.
[67] Zu, X. P., Lin, Z., Xie, H. S., Niao, Y., Liu, X. R., Zhang, W. D. (2016): Interaction of effective ingredients from traditional Chinese medicines with intestinal microbiota. – China Journal of Chinese Materia Medica 41(10): 1766-1772.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2106_59415959

APPENDIX



Appendix 1. *Effects of HFD and baicalin on the body weight of male mice. (A) First week; (B) second week; (C) third week; (D) fourth week; (E) fifth week; (F) sixth week; (G) seventh week; (H) eighth week; (I) ninth week; (J) tenth week; (K) eleventh week. *p < 0.05; **p < 0.01; ***p < 0.001*

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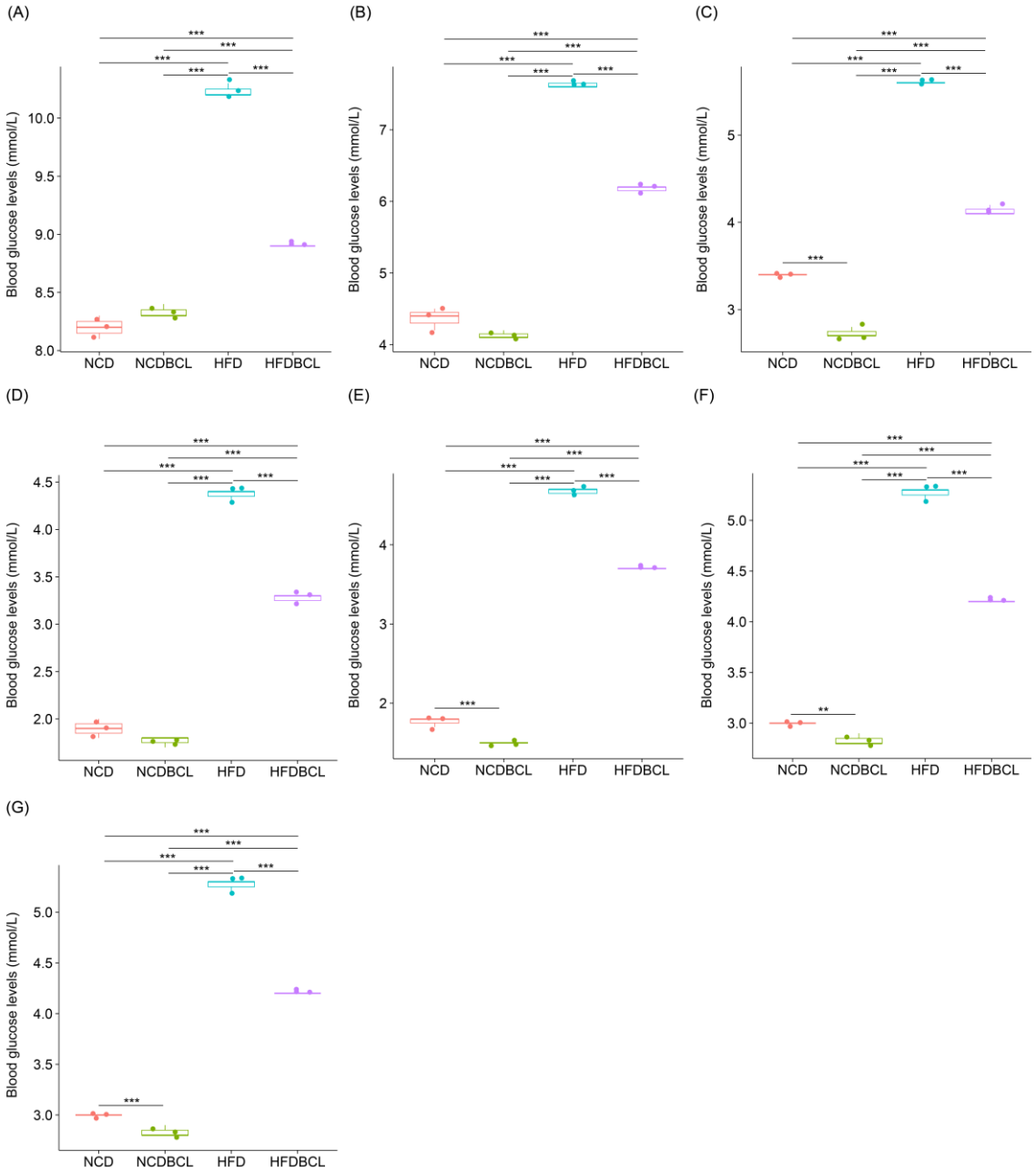

Appendix 2. *Effects of HFD and baicalin on the blood glucose levels of male mice at the end of experiment. (A) Zero min; (B) 15 min; (C) 30 min; (D) 60 min; (E) 90 min; (F) 120 min; (G) 150 min. **p < 0.01; ***p < 0.001*

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