Helicobacter pylori infection exacerbates nonalcoholic fatty liver disease through lipid metabolic pathways: a transcriptomic study.

Xingcen Chen
The Second Xiangya Hospital of Central South University

Ruyi Peng
The Second Xiangya Hospital of Central South University Department of Gastroenterology

Dongzi Peng
The Second Xiangya Hospital of Central South University Department of Gastroenterology

Deliang Liu
The Second Xiangya Hospital of Central South University Department of Gastroenterology

Rong Li
xylulr@csu.edu.cn

The Second Xiangya Hospital of Central South University Department of Gastroenterology
https://orcid.org/0000-0003-2695-1640

Research Article

Keywords: Helicobacter pylori, nonalcoholic fatty liver disease, transcriptome sequencing, FABP5, PPAR signaling pathway

Posted Date: April 17th, 2024

DOI: https://doi.org/10.21203/rs.3.rs-4196201/v1

License: ☺️ This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

Background

The relationship between Helicobacter pylori (H. pylori) infection and nonalcoholic fatty liver disease (NAFLD) have attracted increased clinical attention. However, most of those current studies involve cross-sectional studies and meta-analyses, and experimental mechanistic exploration still needs to be improved. This study aimed to investigate the mechanisms by which H. pylori impacts NAFLD.

Methods

We established two H. pylori-infected (Cag A positive and Cag A negative) mouse models with 16 weeks of chow diet (CD) or high-fat diet (HFD) feeding. Body weight, liver triglyceride, blood glucose, serum biochemical parameters, inflammatory factors, and insulin resistance were measured, and histological analysis of liver tissues was performed. Mouse livers were subjected to transcriptome RNA sequencing analysis.

Results

Although H. pylori infection could not significantly affect serum inflammatory factor levels and mouse liver pathology, serum insulin and homeostatic model assessment for insulin resistance levels increased in CD mode. In contrast, H. pylori infection significantly aggravated hepatic pathological steatosis induced by HFD and elevated serum inflammatory factors and lipid metabolism parameters. Hepatic transcriptomic analysis revealed 767 differentially expressed genes (DEGs) in the H. pylori-infected group in the CD groups, and the "nonalcoholic fatty liver disease" pathway was significantly enriched in KEGG analysis. There were 578 DEGs in H. pylori infection combined with the HFD feeding group, and DEGs were significantly enriched in "fatty acid degradation" and "PPAR pathway." Exploring the effect of different Cag A statuses on mouse liver revealed that fatty acid binding protein 5 was differentially expressed in Cag A- H. Pylori and DEGs enrichment pathways were concentrated in the "PPAR pathway" and "fatty acid degradation."

Conclusions

H. pylori infection may exacerbate the development of NAFLD by regulating hepatic lipid metabolism, and the H. pylori virulence factor Cag A plays a vital role in this regulation.

1. INTRODUCTION

Helicobacter pylori (H. pylori) infects approximately 4.4 billion people worldwide, with a prevalence of 43.1% (40.3–45.9) [1, 2]. A family-based epidemiological survey revealed that the prevalence of H. pylori
infection in China was approximately 40.66%, 43.45% in adults, and 20.55% in children and adolescents [3]. Multitudinous studies have confirmed that *H. pylori* infection is an essential factor in the progression from gastritis to gastric cancer [4, 5]. Cytotoxin-associated gene A (Cag A) is considered the most vital virulence factor of *H. pylori*, and several studies have shown that Cag A is directly associated with DNA damage in gastric epithelial cells and gastric mucosal carcinogenesis [6, 7]. In addition to gastritis, gastric ulcers, and gastric cancer, many extragastric diseases, such as atherosclerosis, Parkinson's disease, and nonalcoholic fatty liver disease (NAFLD), are also closely associated with *H. pylori* infection [8].

NAFLD is defined as a clinicopathologic syndrome characterized by excessive fat deposition in hepatocytes, excluding alcohol and other definite liver-damaging factors. The disease spectrum includes simple hepatocellular steatosis, nonalcoholic steatohepatitis (NASH), NASH-related liver fibrosis, and hepatocellular carcinoma (HCC). The pathogenesis of NAFLD remains unknown, and the *multiple-hit* theory reviewed by Buzzetti et al. is widely acknowledged in academia [9]. NAFLD has become the most common chronic liver disease worldwide, with a global prevalence of approximately 32.4% (29.9–34.9) [10, 11]. Although NAFLD is an urgent public health problem, no country is fully prepared to address it [12]. No effective agents have been approved for NAFLD treatment, and the primary clinical management regimen for NAFLD is to identify patients with a high risk of disease progression and lose weight through dietary modification and physical exercise [10]. It is pressing to recognize and manage NAFLD correctly. Inspiringly, with the specification of the NAFLD definition, the nomenclature for new fatty liver diseases: metabolic dysfunction-associated steatotic liver disease (MASLD) will provide more accurate and high-quality studies for NAFLD/MASLD [13].

Since the first report of *H. pylori* DNA detected in the livers of NAFLD patients [14], several clinical studies have focused on the relationship between *H. pylori* infection and NAFLD. Many scholars discuss the relationship between the two and believe that *H. pylori* infection may be used as a combustion aid in the *multiple-hit* theory of NAFLD, exacerbating the progression of NAFLD through the aspects of inflammatory factors, adipokines, the intestinal barrier, and the intestinal flora [15, 16]. Yu et al. substantiated that eradication of *H. pylori* in *H. pylori*-positive NAFLD patients ameliorated fasting blood glucose (FBG), serum triglycerides (TGs), insulin resistance (IR), and body mass index (BMI) [17]. The study by Abdel-Razik et al. reached similar conclusions [18]. However, other studies have found no association between *H. pylori* infection and NAFLD. A Mendelian randomization study by Liu et al. revealed no causal link between *H. pylori* infection and NAFLD and no significant association between *H. pylori* infection and TGs, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), or FBG levels [19]. Interestingly, a cross-sectional study by Kang et al. indicated that Cag A status may be critical to influencing the relationship between the two, and there was no association between the Cag A positive *H. pylori* group and NAFLD (OR: 1.05; 95% CI: 0.81–1.37), and in multivariate analysis, the Cag A negative (Cag A-) *H. pylori* group was significantly associated with NAFLD (OR: 1.30; 95% CI: 1.01–1.67) [20]. Therefore, this study aimed to explore the effect of *H. pylori* infection with different Cag A status on the liver under different dietary patterns and to explore the relationship between *H. pylori* infection and NAFLD.
2. METHODS

2.1 *H. pylori* culture

The rodent-adapted *H. pylori* Sydney strain (SS1) (Cag A+) was donated by Professor Yong Xie (Department of Gastroenterology, First Affiliated Hospital of Nanchang University, Jiangxi, China). *H. pylori* Cag A- was isolated from gastric ulcer patients’ specimens via gastroscopy. The *H. pylori* strains grown on Columbia blood agar plates supplemented with antibiotics (10 mg/L vancomycin, 5 mg/L ceftulodin, 5 mg/L amphotericin B, and 5 mg/L trimethoprim) and 10% sheep blood (Bianzhen Biotech, Nanjing, China) at 37°C under microaerophilic conditions (5% O\(_2\), 10% CO\(_2\), and 85% N\(_2\)) for 3–4 days. Then, the *H. pylori* strain, which was in the early log phase with good motility and activity for subculture or intervention, was harvested and resuspended in phosphate buffer saline (PBS). The *H. pylori* concentration was estimated by measuring the OD\(_{600\,\text{nm}}\), where OD\(_{600\,\text{nm}}\) corresponds to approximately 2 \(\times\) 10\(^8\) colony-forming units (CFU)/ml.

2.2 Animals and treatment

All animal studies were performed according to the National Institutes of Health recommendations for the Care and Use of Laboratory Animals and were approved by the Central South University Animal Ethics Committee. Male C57BL/6J mice (specific pathogen-free grade) aged 6–8 weeks were purchased from Hunan SJA Laboratory Animal Co., Ltd and housed in animal quarters at 20–22 °C with a 12-h light cycle and fed ad libitum. After one week of adaptive feeding, 48 mice were randomly divided into six groups (PBS, SS1, Cag A-, PBS + HFD, SS1 + HFD, and Cag A-+HFD) of 8 mice each. Four groups were intragastrically infused seven times with 1 \(\times\) 10\(^9\) CFU of *H. pylori* SS1 or *H. pylori* Cag A- at 1-day intervals and fed either a regular chow diet (CD) or a high-fat diet (HFD) (Research Diets, D09100310). Caloric composition of CD versus HFD was shown in Table 1. At the same time, the other two groups received PBS by gavage and were fed the corresponding diet for 16 weeks. The animal experiments were conducted in two parts. The first part focused on examining the impact of *H. pylori* (SS1) infection on mice’s physiological metabolism and liver transcriptomics under different dietary patterns (Results Section 3.1–3.3). The second part investigated the effects of *H. pylori* infection, with different Cag A status, on the liver transcriptomics of mice under different dietary patterns (Results Section 3.4–3.5). Mice were fasted overnight prior to sacrifice. Six animals per group were used for testing and statistical analysis.

<table>
<thead>
<tr>
<th>Dietary component</th>
<th>Protein</th>
<th>Carbohydrate</th>
<th>Fat</th>
<th>Calories</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chow diet</td>
<td>22.9%</td>
<td>66.0%</td>
<td>11.1%</td>
<td>3.37 Kcal/gm</td>
</tr>
<tr>
<td>High fat diet</td>
<td>20.0%</td>
<td>40.0%</td>
<td>40.0%</td>
<td>4.49 Kcal/gm</td>
</tr>
</tbody>
</table>

2.3 Biochemical analysis
Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), total cholesterol (TC), HDL-C, and LDL-C were assayed by an automatic biochemical analyzer (Rayto Life and Analytical Sciences, Chemray 240) with corresponding commercial kits. Enzyme-linked immunosorbent assay (ELISA) kits (Jiangsu Meimian Industrial, MM-0040M1, MM-0163M1, MM-0132M1, and MM-0579M1) were used to detect Interleukin 1β (IL-1β), Interleukin 6 (IL-6), Tumor necrosis factor α (TNF-α) and insulin levels in mouse serum. Hepatic TGs were measured by a commercial kit (Nanjing Jiancheng Bioengineering Institute, A110-1-1) according to the manufacturer’s instructions.

2.4 Histopathologic examination

Fresh mouse liver tissues were fixed in 4% paraformaldehyde and embedded in paraffin. Embedded tissues were cut at 4 µm thickness and then stained with a hematoxylin-eosin (H&E) kit (Powerful Biology, Wuhan, China) for histological assessment according to the rodent model NAFLD scoring system proposed by Liang et al. [21]. Frozen samples were cut into 8-µm sections and stained with an Oil Red O staining kit (Powerful Biology, Wuhan, China) according to the manufacturer’s instructions. Masson staining was used to observe fibrosis in the liver of mice. After staining with iron hematoxylin, Ponceau, and aniline blue, collagen fibers were blue, and muscle fibers, cytoplasm, and cellulose were red. Immunohistochemical (IHC) staining was performed using the following methods: Paraffin slices, 4 µm thick, were grilled at 65°C for 60 minutes, then dewaxed in xylene and rehydrated in a series of increasingly diluted ethanol. High-temperature antigen retrieval was achieved by microwave treatment in 0.1 M citrate solution (pH 6.0) for 10 minutes. The slices were treated with 3% H₂O₂ at room temperature for 20 minutes, followed by incubation with goat serum for 20 minutes, and subsequently with anti-FABP5 rabbit polyclonal antibody (Proteintech, Wuhan;1:100) overnight at 4°C. The following day, the slices were brought to room temperature and incubated with the secondary anti-rabbit antibody for 20 minutes after washing with PBS. DAB coloration was applied, followed by mounting with hematoxylin and subsequent microscopic examination. All the samples were examined under a light microscope at 200× magnification.

2.5 Intraperitoneal glucose tolerance test (IPGTT) and intraperitoneal insulin tolerance test (IPITT)

In the 15th week of intervention, the mice were pre-stimulated for approximately one week. Sixteen hours of fasting and water deprivation preceded the IPGTT procedure, and the mice were injected intraperitoneally with glucose solution (2 g/kg body weight). Blood samples were collected by tail puncture at 0, 30, 60, 90, and 120 min to measure blood glucose levels using a glucometer. The area under the curve (AUC) was calculated for the IPGTT. IPITT was performed at three-day intervals. Mice were fasted for six hours and injected with insulin (0.75 IU/kg body weight) (Jiangsu Wanbang Biochemistry Medicine Co. Ltd., Xuzhou, China). Blood glucose levels were measured at 0, 15, 30, 60, and 90 min after insulin injection. The AUC of the IPITT was calculated.

2.6 RNA-sequencing
Total RNA preparation and subsequent RNA-seq library construction were performed using the APExBIO Technology LLC (Shanghai, China) service. Briefly, total RNA was isolated using a commercial kit (Tiangen Biotech, DP424), and RNA libraries were established using an RNA cleaning and concentration kit (APExBIO Technology LLC, K1159) after quality inspection and purity testing. The RNA quality was verified using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). The qualified libraries were subjected to Illumina NovaSeq 6000 double-end sequencing according to the effective concentration and target data volume to obtain paired sequences with a read length of 150 bp. The filtered reads were mapped to the mouse genome reference sequence (GRCm39.dna.toplevel.fa Ensembl release103) using HISAT2. The gene expression levels are expressed as fragments per kilobase per million fragments (FPKM). Genes were considered differentially expressed when |fold change| >1.5 and P value < 0.05. Differential expression analysis was performed using DESeq2. KEGG and GO enrichment analyses of differentially expressed genes were performed using the R package ClusterProfiler (v4.2.2), and significant pathways were identified with a P value < 0.05.

### 2.7 Quantitative real-time PCR (qRT-PCR)

Total RNA was extracted from the livers with AG RNAex Pro Reagent (Accurate Biotechnology, AG21101), an Evo M-MLV RT Mix Kit with gDNA Clean for qPCR Ver.2 (Accurate Biotechnology, AG11728) reverse-transcribed the extracted total RNA into cDNA. qRT-PCR was performed using the SYBR Green Premix Pro Taq HS qPCR Kit IV (Accurate Biotechnology, AG11746) and the targeting gene primers. All the gene primer sequences are shown in Table 2. PCR was performed in triplicate on the qRT-PCR detection system with the following cycling parameters: 95°C (30 s), 40 cycles of 95°C (5 s), 55°C (30 s), and 72°C (30 s). The qRT-PCR data were quantified by the $2^{-\Delta\Delta Ct}$ method.

Table 2

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward primer</th>
<th>Reverse primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gapdh</td>
<td>AGGTCGGTGTGAACGGATTTG</td>
<td>GGGGTCGTGGATGGCAACA</td>
</tr>
<tr>
<td>Fabp5</td>
<td>AGAGCACAGTGAAAGACGAC</td>
<td>CATGACACACTCCACGATCA</td>
</tr>
<tr>
<td>Ppar-γ</td>
<td>TCGCTGATGCACTGCCTATG</td>
<td>GAGAGGTCCACAGAGTCTATT</td>
</tr>
<tr>
<td>Fgf21</td>
<td>CTGCTGGGGGTCTACCAAG</td>
<td>CTGCGCCTACCACGTTCC</td>
</tr>
<tr>
<td>Srebf-1</td>
<td>GATGTCGCAACTGAGCACAG</td>
<td>CATAGGGGGCGGTCAACAG</td>
</tr>
<tr>
<td>Il-1β</td>
<td>GAAATGCCACCTTTTGACAGT</td>
<td>TGGATGCTCTCATCAGGACAG</td>
</tr>
<tr>
<td>Tnf-α</td>
<td>CCTGTAGCCCCACGTCGTA</td>
<td>GGGAGTAGAACAAGGTACAAAC</td>
</tr>
</tbody>
</table>

### 2.8 Statistical analysis

The data are expressed as the mean ± standard deviation (SD) and analyzed by unpaired student’s t-test and one-way analysis of variance (ANOVA) with GraphPad Prism 9.0 software. At least three independent
biological replicates were performed for each group. $p < 0.05$ was considered statistically significant.

3. RESULTS

3.1 *H. pylori* infection exacerbates hepatic lipid deposition and insulin resistance in mice

Mice infected with *H. pylori* had a slightly lower body weight curve than non-infected mice under CD conditions. Although there was no statistical difference between the two groups, infected mice showed an increasing trend in liver weight and liver weight ratio compared with non-infected mice. TG content in the liver of *H. pylori*-infected mice was significantly higher than that of non-infected mice (Supplementary Fig. 1A-D). Histopathological observations of the liver are shown in Fig. 1A, and the semiquantitative score of NAFLD also tended to be higher in mice infected with *H. pylori* (Fig. 1B).

In the HFD setting, the trend of physiological parameters in *H. pylori*-infected mice was similar to that in the CD groups. The weight curve of *H. pylori*-infected mice was slightly lower than that of non-infected mice, and the liver weight and liver weight ratio of infected mice tended to increase (Supplementary Fig. 1E-H). Notably, liver pathological changes were more prominent in mice on an HFD, and *H. pylori*-infected mouse livers had more pronounced hepatocyte damage, such as hepatocyte macrovesicular steatosis and hepatocyte swelling (Fig. 1C). Thus, there were significant differences between *H. pylori*-infected mice and non-infected mice in hepatic TG content and NAFLD score (Fig. 1D).

IPGTT and IPITT tested glucose homeostasis and insulin sensitivity. Interestingly, *H. pylori* infection affects mice glucose regulation ability and insulin resistance in the CD groups. In the CD groups, the AUC of IPGTT, IPITT, was significantly higher in *H. pylori*-infected mice than in the non-infected group. Serum insulin levels and IR levels were also higher in the infected group than in the non-infected group (Supplementary Fig. 2). In the HFD feeding, glucose and insulin regulation were weaker, and serum insulin levels were higher in *H. pylori*-infected mice than in non-infected mice. However, IR scores were not significantly different between the two groups (Supplementary Fig. 2).

3.2 *H. pylori* infection combined with HFD feeding had the most significant effect on serum metabolism and inflammation in mice

Serum LDL-C in *H. pylori*-infected mice in the CD groups was statistically different from that in the non-infected mice. However, HDL-C, TC, and serum liver enzymes (ALT, AST) showed no significant difference. Serum inflammatory cytokines IL-1β, IL-6, and TNF-α were slightly increased in *H. pylori*-infected mice (Fig. 2).

In contrast, *H. pylori* infection combined with HFD feeding had a more pronounced effect on serum biochemical parameters in mice. Although there was no statistical difference in serum liver enzymes between the two groups, serum TC, LDL-C, and serum inflammatory cytokines IL-6 and TNF-α were significantly higher in the *H. pylori*-infected group than those in the non-infected group (Fig. 2).
3.3 RNA-seq reflects different gene expression profiles in the liver of *H. pylori*-infected mice fed with different dietary patterns

Differential expression genes (DEGs) were identified as | fold changes | > 1.5, with a P value < 0.05. Through the analysis of the RNA transcriptome sequencing results of the liver of mice in the CD groups, 767 DEGs were present in the liver of mice in the *H. pylori*-infected group (Fig. 3A), including 396 down-regulated genes and 371 up-regulated genes. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses to identify the biological processes associated with the DEGs. Visualization of the DEGs enriched functional results showed that the "fatty acid metabolic process" was significantly expressed (Fig. 3B). The enrichment results significantly expressed the "Nonalcoholic fatty liver disease" pathway in the KEGG enrichment analysis (Fig. 3C). In GO enrichment analysis, the top 3 significantly enriched in the biological process were "cellular process," "metabolic process," and "biological regulation (Fig. 3D)."

Analysis of the RNA transcriptome sequencing results of the liver of mice in the HFD groups showed that there were a total of 578 DEGs in the liver of mice infected with *H. pylori* (Fig. 4A), including 245 down-regulated genes and 333 up-regulated genes. Visualization of the DEGs enriched functional results showed that "long-chain fatty acid metabolic process" and "regulation of lipid metabolic process " were significantly expressed (Fig. 4B). In KEGG enrichment analysis, the "PPAR signaling pathway," "Fatty acid degradation," and "Retinol metabolism" pathways were significantly expressed in the enrichment results (Fig. 4C). The GO enrichment analysis results were similar to those of the CD groups, and the top three biological processes were "cellular process," "metabolic process," and "biological regulation (Fig. 4D)."

3.4 Differential gene expression profiles in livers of *H. pylori*-infected mice with different Cag A status on a uniform diet

In the CD pattern, there were 1511 DEGs in Cag A- *H. pylori* infection compared with Cag A+ *H. pylori* infection (Fig. 5A), of which 780 were up-regulated, and 731 were down-regulated. Fatty acid binding protein 5 (Fabp5), a critical intracellular transporter of fatty acid and a key regulator of the PPAR pathway, was significantly up-regulated in the Cag A- group. The visualized DEG enrichment function results showed that the "fatty acid metabolic process" and "fat catabolic process" were significantly expressed (Fig. 5B). The "PPAR signaling pathway" and "Fatty acid degradation" pathways were enriched in KEGG enrichment analysis. GO enrichment analysis results are shown in Fig. 5D.

In HFD feeding, there were 1400 DEGs in Cag A- *H. pylori* infection compared with Cag A+ *H. pylori* infection (Fig. 6A), of which 762 were up-regulated and 638 were down-regulated. Fabp5 was stably and highly expressed in this group. The visualized DEGs enrichment function results showed that "fatty acid oxidation" and "long-chain fatty acids" were significantly expressed (Fig. 6B). The KEGG enrichment analysis enriched the "PPAR signaling pathway" and "Fatty acid degradation" pathways. GO enrichment analysis results are shown in Fig. 6D.
3.5 qRT-PCR and IHC verified the expression level of differentially expressed genes in mouse liver

Subsequently, qRT-PCR results showed that *H. pylori* infection significantly increased the expression levels of sterol regulatory element binding transcription factor 1 (Srebf1), fibroblast growth factor 21 (Fgf21), the critical factors of lipid metabolism, Il-1β, and Tnf-α in the liver (Fig. 7A-D). We performed qRT-PCR to validate the transcriptome sequencing DEGs, and the validation results are shown in Fig. 7E, F. Meanwhile, for the most significantly differentially expressed Fabp5, we performed IHC staining of the mouse liver. Fabp5 expression levels were lower in Cag A+ *H. pylori*-infected mouse livers. In contrast, cells with strong positive Fabp5 expression displayed in Cag A- *H. pylori*-infected mouse livers, mainly distributed in interstitial tissues. The results were shown in Fig. 7G.

4. DISCUSSION

We established mouse models of *H. pylori* infection under different dietary patterns to investigate the association between *H. pylori* infection and NAFLD. In the CD groups, TG content in liver tissue, serum insulin, and HOMA-IR scores demonstrated that *H. pylori* infection could cause hepatic TG deposition and insulin resistance in mice. However, serum biochemical parameters, liver enzymes, and liver pathology indicated that *H. pylori* infection does not significantly affect mice's physiological metabolism. We suspected the possible reasons may be: (1) *H. pylori* infection duration is not long enough, the systemic chronic inflammatory response is not apparent, and (2) *H. pylori* infection alone is insufficient to produce significant changes in liver pathology. Analysis of the liver transcriptome sequencing results showed that *H. pylori* infection induced 767 DEGs in mouse liver tissues, of which 371 genes were up-regulated, and 396 genes were down-regulated. Enrichment analysis showed that some DEGs were significantly involved in the "fatty acid metabolism" and "non-alcoholic fatty liver disease" pathway. These transcriptome results illustrate the link between *H. pylori* infection and NAFLD.

Based on HFD feeding, *H. pylori* infection had a more evident effect on the physiological metabolism of mice. Although there was no statistical difference in body weight and liver weight between the two groups, liver TG content, serum TC, LDL-C, and serum inflammatory cytokines IL-6 and TNF-α were significantly higher in the *H. pylori*-infected group than in the non-infected group. *H. pylori* infection combined with HFD feeding resulted in more significant hepatic lipid deposition and hepatocyte macrovesicular steatosis in mice, and there were significant differences in NAFLD scores in mice, which coincided with the findings of He et al. [22]. At the same time, mice infected with *H. pylori* showed decreased sensitivity to glucose and insulin. Analysis of liver transcriptome sequencing results showed that *H. pylori* infection under HFD feeding conditions induced differential expression of 578 genes in mouse liver tissues, of which 245 genes were up-regulated and 333 genes were down-regulated. Enrichment analysis found that some DEGs were significantly involved in the "long-chain fatty acid metabolic process" and "regulation of lipid metabolic process." Meanwhile, "Retinol metabolism" and
"PPAR signaling pathway" were significantly enriched in KEGG analysis. The results of these analyses illustrate that HFD-based *H. pylori* infection impacts hepatic lipid metabolism.

By comparing the effect of *H. pylori* strain infection with different Cag A status on liver transcriptomics under the uniform dietary pattern, we explored the possible role of the virulence factor Cag A in the relationship between *H. pylori* infection and NAFLD. The comparison revealed that the "PPAR signaling pathway" and "Fatty acid degradation" pathways were significantly enriched in DEGs from livers of Cag A- *H. pylori*-infected mice regardless of dietary pattern. In addition, Fabp5 was upregulated in the transcriptome DEGs, a critical regulator of lipid metabolism.

Several experimental studies have explored the relationship between *H. pylori* and NAFLD directly. He et al. reported that *H. pylori* infection combined with 12 weeks of HFD feeding promoted central obesity and IR in mice to a comparable extent as HFD feeding alone for 24 weeks, and dynamic changes in the gut microbiota may cause these effects [22]. Subsequently, the authors measured hepatic lipid deposition in the liver, and NAFLD scores revealed that *H. pylori* infection significantly aggravated HFD-induced NAFLD and different *H. pylori* strains, most notably SS1, had different exacerbating effects on NAFLD [23]. Notably, the *H. pylori* strains used in the above studies (SS1 and NCTC 11637) did not include the Cag A-strain, and we established a Cag A-strain control in combination with clinical epidemiological studies and *H. pylori* virulence factor studies to explore the effects of different *H. pylori* strains further. In addition, *H. pylori* infection has been demonstrated to promote CCl₄-induced liver fibrosis in animal models [24]. In this study, it was possible that HFD plus *H. pylori* infection only intervened for 16 weeks, and no significant hepatic fibrosis was observed via Masson staining of liver sections. Combined with the reported literature [25], we estimated that HFD feeding alone requires at least 24 weeks to visualize significant fibrosis in the livers of mice.

Previous studies have confirmed that Cag A is closely related to the occurrence of gastric cancer. Reports on Cag A combined with extragastric diseases are common in patients with atherosclerosis [26, 27], and only two studies have reported the association between Cag A and NAFLD. Kang et al. suggested that the *H. pylori* Cag A-strain may be associated with NAFLD [20]. In contrast, Barreyro et al. reported no significant association between *H. pylori* infection, Cag A status, and ultrasonographically diagnosed NAFLD in NAFLD patients with dyspeptic symptoms [28]. Moreover, the results suggested that Cag A+ but not Cag A- was associated with higher AST and fibrosis 4 scores in patients. Our study is the first transcriptomical research to mechanistically explore the relationship between Cag A, *H. pylori*, and NAFLD. Sequencing analysis of liver transcriptomes infected with different *H. pylori* strains revealed that “Nonalcoholic fatty liver disease” and “PPAR signaling pathway” were enriched according to KEGG enrichment analysis, and Fabp5 expression was significantly different in the Cag A- groups.

Fabp5 is a member of the fatty acid binding protein family, which is mainly involved in the uptake, transport, and metabolism of fatty acids and related metabolites in the cytoplasm and regulating lipid metabolism and cell growth [29]. Fabp5 is essential for the pathogenesis of IR associated with obesity and lipid metabolism [30, 31]. Loss of Fabp5 gene expression leads to increased systemic insulin
sensitivity in animal models of obesity and IR, and adipocytes isolated from Fabp5 -/- mice also exhibit increased insulin-stimulated glucose transport capacity [30]. In contrast, mice with high Fabp5 expression in adipose tissue exhibited significantly decreased systemic insulin sensitivity, and Fabp5 may regulate blood glucose and blood lipid metabolism by affecting leptin expression.

In this study, we detected the differential expression of Fabp5 in each group by qRT-PCR. Interestingly, Fabp5 was highly expressed in both the Cag A- groups but not in the Cag A + groups, regardless of the dietary ingredient. In addition, two bioinformatics studies predicted the crucial role of Fabp5 in NAFLD. High Fabp5 expression was significantly associated with poor prognosis in NAFLD-related HCC patients [32, 33]. These results fit our results to some extent. Therefore, we speculated that other virulence factors of H. pylori, such as vacuolating toxin A, neutrophils activating protein, upregulated Fabp5 expression through some mechanisms, while the presence of Cag A, the most potent virulence factor, masked the mechanisms. However, additional experimental studies are needed to explore the underlying mechanisms of H. pylori virulence factors and extragastric diseases such as NAFLD.

The enrichment analysis results suggested that the retinol metabolic and PPAR signaling pathways were significantly enriched in the HFD groups. Retinol and its primary metabolites, retinal and all-trans retinoic acid (atRA), are collectively referred to as naturally occurring retinoids, which control energy balance, obesity, and inflammatory processes. Total cellular reflectance retinoic acid binding protein (CRABP) is the primary receptor for intracellular retinoid transport, and Fabp5 also has a high affinity for atRA and long-chain fatty acids. Fabp5 competitively binds atRA with CRABP2, and when the Fabp5/CRABP2 ratio is high, atRA binds Fabp5 and activates the downstream PPAR pathway, a crucial pathway regulating glucose and lipid metabolism [34, 35]. We speculate that the overexpression of Fabp5 in mouse hepatocytes caused by H. pylori infection inhibits CRABP2, binds to atRA for transport, activates the downstream PPAR pathway, and, in turn, regulates fatty acid degradation pathways. Our experimental results concatenate H. pylori infection exacerbating NAFLD into a complete clue.

5. CONCLUSION

In summary, we established a mouse model of NAFLD plus H. pylori infection and found that chronic H. pylori infection significantly aggravated HFD-induced hepatic lipid deposition and IR. Through transcriptome sequencing analysis and related validation, we discovered that H. pylori infection may promote the development of NAFLD by regulating lipid metabolism. However, how does H. pylori infection regulate Fabp5 expression in the liver, through hepatic macrophages? H. pylori exoteric vesicles? Or other pathways. Basic experiments are needed to explore the underlying mechanisms involved in NAFLD, which will help us better comprehend NAFLD and gain insight into the pathways through which H. pylori causes extragastric diseases.

Abbreviations

H. pylori: Helicobacter pylori
**NAFLD**: Nonalcoholic fatty liver disease

**Cag A**: Cytotoxin-associated gene A

**Fabp5**: Fatty acid binding protein 5

**PPAR**: Peroxisome proliferator-activated receptor

**IR**: Insulin resistance

**HOMA-IR**: Homeostatic model assessment for insulin resistance

**FBG**: Fasting blood glucose

**TG**: Triglyceride

**BMI**: Body mass index

**HDL-C**: High-density lipoprotein cholesterol

**LDL-C**: Low-density lipoprotein cholesterol

**HFD**: High-fat diet

**CD**: Chow diet

**ALT**: Alanine aminotransferase

**AST**: Aspartate aminotransferase

**TC**: Total cholesterol

**IPGTT**: Intraperitoneal glucose tolerance test

**IPITT**: Intraperitoneal insulin tolerance test

**KEGG**: Kyoto Encyclopedia of Genes and Genomes

**GO**: Gene Ontology

**DEG**: Differentially expressed gene

**IL-1β**: Interleukin 1β

**TNF-α**: Tumor necrosis factor α

**IL-6**: Interleukin 6
atRA: All-trans retinoic acid

Declarations

Ethics approval and consent to participate

The animal studies were performed according to the National Institutes of Health recommendations for the Care and Use of Laboratory Animals and were approved by the Central South University Animal Ethics Committee.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests

Funding

This work was supported by the National Natural Science Foundation of China (Grant No. 82070547) and the Natural Science Foundation of Hunan Province (Grant Nos. 2024JJ5492).

Authors’ contributions

CXC performed the research, contributed to the analysis and wrote the paper; PRY and PDZ wrote the paper and supervised the research; LDL supervised the research and revised the manuscript; and LR designed the research, supervised the research and revised the manuscript. All the authors read and approved the final manuscript.

Acknowledgements

Not applicable.

References


**Figures**

**Figure 1**

Effects of *H. pylori* infection combined with CD/HFD feeding on liver pathology in mice. (A) Gross observation, HE staining, Masson staining and Oil Red O staining in CD groups, 200× magnification under a light microscope. (B) Semi-quantitative NAFLD score by HE staining in CD groups. (C) Gross observation, HE staining, Masson staining and Oil Red O staining in HFD groups, 200× magnification under a light microscope. (D) Semi-quantitative NAFLD score by HE staining in HFD groups. Data are expressed as mean ± SD, n=6, *P<0.05, **P<0.01, ***P<0.001.
Figure 2

Effects of *H. pylori* infection combined with CD/HFD feeding on physiological metabolism in mice. (A) Serum total cholesterol (TC, mmol/L). (B) Serum high-density lipoprotein cholesterol (HDL-C, mmol/L). (C) Serum low-density lipoprotein cholesterol (LDL-C, mmol/L). (D) Serum alanine aminotransferase (ALT, U/L). (E) Serum aspartate aminotransferase (AST, U/L). Serum inflammatory factor: (F) IL-1 beta (pg/mL). (G) IL-6 (pg/mL). (H) TNF-α (pg/mL). Data are expressed as mean ± SD, n=6, *P<0.05, **P<0.01, ***P<0.001.
Figure 3

PBS vs *H. pylori* transcriptomic DEGs analysis. (A) volcano plot of DEGs; (B) DEGs enrichment results visualization, circular cnetplot; (C) KEGG enrichment analysis; and (D) GO enrichment analysis.
Figure 4

PBS+HFD vs H. pylori+HFD transcriptomic DEGs analysis. (A) volcano plot of DEGs; (B) DEGs enrichment results visualization, circular cnetplot; (C) KEGG enrichment analysis; and (D) GO enrichment analysis.
Figure 5

Cag A+ vs Cag A- transcriptomic DEGs analysis. (A) volcano plot of DEGs; (B) DEGs enrichment results visualization, circular cnetplot; (C) KEGG enrichment analysis; and (D) GO enrichment analysis.
Figure 6

HFD+Cag A+ vs HFD+Cag A- transcriptomic DEGs analysis. (A) volcano plot of DEGs; (B) DEGs enrichment results visualization, circular cnetplot; (C) KEGG enrichment analysis; and (D) GO enrichment analysis.
Figure 7

qRT-PCR and IHC validated DEGs expression in mouse liver. Critical lipid metabolism factor Srebf (A), Fgf21 (B) inflammatory factor Il-1β (C), Tnf-α (D). DEGs Fabp5, PPAR-γ were expressed in mouse liver (E, F). Results of Fabp5 IHC staining in mouse liver in each group (G), 200× magnification under a light microscope. Data are expressed as mean ± SD, n=6, *P<0.05, **P<0.01, ***P<0.001.
Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarymaterials.docx