



Research Paper

Helicobacter pylori Infection Aggravates Diet-induced Insulin Resistance in Association With Gut Microbiota of Mice



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ABSTRACT

Emerging evidence suggests that *Helicobacter pylori* infection is associated with insulin resistance (IR) yet the underlying mechanisms are still obscure. The vital role of gut microbiota in triggering IR has been increasingly reported, however, no study has explored the correlation of gut microbiota and *H. pylori*-associated IR. Using *H. pylori*-infected mice model fed different diet structures, we demonstrated that *H. pylori* infection significantly aggravated high-fat diet (HFD)-induced metabolic disorders at the early stage, the extent of which was close to the effect of long-term HFD. Interestingly, we observed dynamic alterations in gut microbiota that were consistent with the changes in the metabolic phenotype induced by *H. pylori* and HFD. There may be an interaction among *H. pylori*, diet and gut microbiota, which dysregulates the host metabolic homeostasis, and treatment of *H. pylori* may be beneficial to the patients with impaired glucose tolerance in addition to diet control.

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1. Introduction

It has been established that *Helicobacter pylori* infection can lead to the development of many gastroduodenal diseases (Goh et al., 2011). Accumulated evidence has suggested that this infection is associated with metabolic diseases, such as metabolic syndrome (Chen et al., 2015a; Chen et al., 2015b), type 2 diabetes mellitus (T2DM) (He et al., 2014b) and atherosclerotic diseases (He et al., 2014a), yet the underlying mechanism is obscure. Insulin resistance (IR) and subsequent hyperinsulinemia are the key pathogenic factors contributing to these metabolic abnormalities. Thus, investigating the impact of *H. pylori* infection on insulin sensitivity may elucidate the association between *H. pylori* and the metabolic diseases.

Increasing evidence has shown that the gut microbiota play a fundamental role in the pathogenesis of metabolic disorders like obesity and T2DM, possibly through modulating energy balance, glucose metabolism and chronic inflammatory response (Cani et al., 2008; Ley et al., 2006; Musso et al., 2010). Thus far, the interaction of gastric microbiota with *H. pylori* in human and animal models has been investigated, and several of these microbes are supposed to contribute to *H. pylori*-associated diseases such as gastric cancer (He et al., 2016). However,

studies regarding the impact of *H. pylori* infection on gut microbiota are limited. In this study, we investigated the effect of *H. pylori* infection on high-fat diet (HFD) induced IR and the associated alterations of gut microbiota in C57BL/6 mice. We also assessed the levels of metabolic hormones and cytokines in response to *H. pylori* colonization with or without a HFD.

2. Material and Methods

2.1. Animals and *H. pylori* Strains

Six- to eight-week-old male C57BL/6 mice (Hunan Slac Jingda Laboratory Animal Company, Ltd., Changsha, China) and two mouse-adapted *H. pylori* strains including the Sydney strain (SS1) and NCTC11637 were used in this study. The *H. pylori* strains were inoculated on Campylobacter agar plates containing 10% sheep blood and incubated at 37 °C under microaerophilic conditions (5% O₂, 10% CO₂, and 85% N₂) for 24–48 h and then subcultured in Brucella broth supplemented with 10% FBS at 37 °C under the microaerophilic conditions for 16–18 h. Bacterial density was estimated spectrophotometrically at 600 nm (OD₆₀₀), and viable cells were determined as colony-forming units (CFU)/ml (1 OD₆₀₀ = 10⁹ CFU/ml). Animals were housed in a controlled environment (12 h daylight cycle) with food and water ad libitum. After 2 weeks of acclimation on a normal chow diet (Beijing KeAoXieLi Company, Ltd., Beijing, China), the mice were randomly

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divided into six groups (Supplement Fig. 1). Four groups were received gavage in a dosage of 1×10^9 CFU of *H. pylori* SS1 or NCTC11637 five times at 2 day intervals, and concurrently fed either a chow diet or HFD containing 45.37% lipids, 18.21% proteins and 36.42% carbohydrates, whereas the other two groups received the sterile Brucella broth and were fed the corresponding diet. Body weight, food intake and waist circumference were assessed twice a month. The waist circumference was measured at the midpoint between the lower ribs and iliac crest with a measuring tape by two investigators who were blinded as to which group the animal has been assigned (Knigge et al., 2015; Zhang et al., 2007). All procedures were approved by the Ethics Committee of The First Affiliated Hospital of Nanchang University.

2.2. Glucose Homeostasis

After 12 and 24 weeks on different diets, mice were fasted overnight (12 h) and the glucose tolerance test (IPGTT) was performed after the mice were intraperitoneally injected with glucose (2 g/kg). Blood glucose concentrations were measured using a handheld glucometer (OneTouch Ultra Easy, LifeScan) via tail bleed before (0 min) and after (15, 30, 60, 120 min) the glucose injection. For the intraperitoneal insulin tolerance test (IPITT), mice were fasted for 6 h and the glucose levels were measured before (0 min) and after (15, 30, 60, 120 min) insulin (0.75 U/kg) administration.

2.3. Blood Serum Analysis

At week 12 and 24, mice were fasted overnight and sacrificed for subsequent analysis. Blood was collected into microfuge tubes and allowed to clot for 30 min. Then samples were centrifuged at 3000 rpm for 20 min and serum was collected and stored at -80°C until analysis. Serum insulin (CrystalChem Inc.) was quantified by ELISA. The homeostasis model assessment of insulin resistance (HOMA-IR) index was calculated based on the following formula: fasting insulinemia ($\mu\text{U/ml}$) \times fasting glycaemia (mM)/22.5. The concentrations of inflammatory cytokines IL-1 β , IL-6 and TNF- α were quantified using the Mouse Cytokine/Chemokine Magnetic Bead Panel (Millipore). The Milliplex MAP Kit for Mouse Metabolic Magnetic Bead Panel (Millipore) was used to quantify hormones including resistin, gastric inhibitory polypeptide (GIP) and leptin.

2.4. 16S rRNA Gene Sequencing and Analysis

Fresh faeces were collected at different time points and total genomic DNA from each sample was extracted using the QIAamp DNA Stool Mini Kit (QIAGEN) according to the manufacturer's instructions. DNA concentrations and integrity were assessed using a fluorometer and agarose gel electrophoresis. Then, the qualified DNA samples were stored at -80°C prior to 16S ribosomal RNA (rRNA) gene amplicon sequencing. The 16S rRNA gene amplicon sequencing was performed on the Illumina MiSeq platform (BGI Tech Solutions Co., Ltd., Shenzhen, China) according to protocols described by Caporaso et al. (2012). The sequences of the primers targeting the V4 hyper-variable region of the bacterial 16S rRNA genes were as follows: 515F GTGCCAGC MGCCGCGGTAA; 806R GGACTACHVGGGTWTCTAAT. The raw data were filtered to obtain clean reads by eliminating the adapter pollution and low quality sequences (Fadrosh et al., 2014). High quality paired-end reads were combined to tags with an average read length of 252 bp using FLASH (Fast Length Adjustment of Short reads, v 1.2.11) (Magoc and Salzberg, 2011). The tags were then clustered as OTU (Operational Taxonomic Unit) by scripts of USEARCH (v 7.0.1090) software with a 97% similarity threshold (Edgar, 2013). The representative OTU sequences were taxonomically classified using Ribosomal Database Project (RDP) Classifier v.2.2 trained on the Greengenes database. Finally, an OTU table and a phylogenetic tree were generated for diversity analysis. Principal coordinate analysis (PCoA) was conducted according to

the distance matrices created by QIIME (v 1.80) (Caporaso et al., 2010). Linear discrimination analysis coupled with effect size (LEfSe) was performed to identify the bacterial taxa differentially represented between groups at the genus or higher taxonomy levels (Segata et al., 2011).

2.5. Western Blotting

10 mg of liver and jejunum tissue were homogenized using the Mixer Mill MM 400 (Retsch) in lysis buffer (50 mM Tris-HCl, pH 7.8, 50 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM Na_3VO_4 , 50 mM β -glycerol-phosphate, 1 mM DTT, 0.5% TritonX-100, 10 mM NaF and protease inhibitors). Protein concentrations were determined using a Coomassie brilliant blue staining method. An equal amount of protein (20–25 μg) was separated on 10% SDS-PAGE gels and transferred to nitrocellulose membranes (Whatman GmbH, Dassel, Germany). After the membranes were blocked with 5% nonfat milk in Tris-buffered saline (TBS) containing 0.1% Tween-20 (TBST) at room temperature for 1 h, they were incubated overnight with the primary antibody at 4°C . Subsequently, the membranes were washed and incubated with HRP-conjugated secondary antibody. Immuno-reactive proteins were detected using an ECL kit (Bio-Rad, USA). Antibodies against protein kinase B (Akt) and p-Akt (Ser473) were purchased from Cell Signaling Technology, insulin receptor substrate 1 (IRS1) and p-IRS1 (Tyr896) and occludin from Abcam, ZO-1 from Invitrogen and claudin-1 from Life Technologies.

2.6. Statistical Analysis

Statistical analysis was performed using the SPSS 13.0 software. Data are expressed as the means \pm standard error of means (SEM). Groups were compared by one-way ANOVA and LSD's multiple range test with a value of $p < 0.05$ as the cut-off for statistical significance.

3. Results

3.1. *H. pylori* Infection Accelerates Diet-induced Central Obesity

The C57BL/6 mice were successfully infected with *H. pylori* after 12 and 24 weeks, which was confirmed by Giemsa staining while none of the mice administered Brucella broth alone was positive for *H. pylori* (Supplement Fig. 2). As shown in Fig. 1A and B, both the body weight and the waist circumference were significantly increased early after HFD feeding. Interestingly, HFD-fed mice infected with *H. pylori* SS1 had a larger waist circumference without increased body weight compared to that of the HFD controls. Moreover, the visceral fat, especially the epididymal adipose mass, was much heavier and the relative epididymal fat pad weight was significantly greater in mice with *H. pylori* infection after 12 weeks of HFD, the degree of which was almost comparable to the level of 24 weeks HFD controls (Fig. 1C and D). Additionally, in contrast to the HFD control group, *H. pylori*-infected mice feeding HFD showed no difference of energy intake in comparison with the controls but exhibited a significant increase in energy efficiency at 12 weeks (Fig. 1E and F). Together, these data suggest that *H. pylori* infection accelerates HFD-induced fat redistribution and the emergence of central obesity which may be related to the enhanced energy harvest.

3.2. *H. pylori* Infection Aggravates Early Diet-induced IR

We next examined the impact of *H. pylori* infection on glucose homeostasis and insulin sensitivity in mice feeding a normal diet or HFD after 12 and 24 weeks. We found that *H. pylori*-infected HFD-fed mice displayed more severe IR compared to HFD controls at 12 weeks as revealed by intraperitoneal insulin tolerance tests (IPITTs) (Fig. 2A). Although *H. pylori* infection did not significantly influence HFD-induced glucose intolerance (Fig. 2B), the higher fasting hyperinsulinemia and

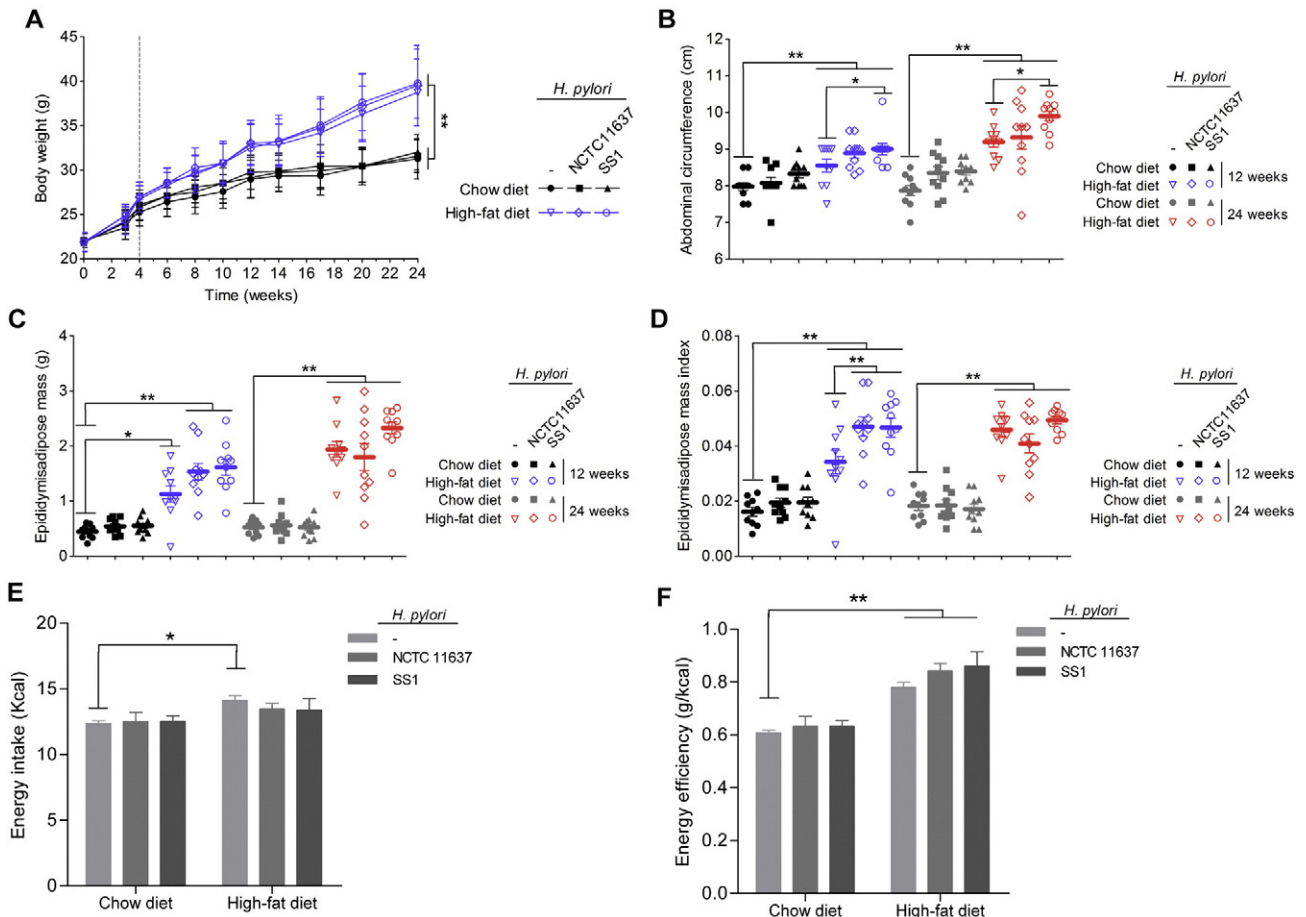


Fig. 1. Effects of *H. pylori* infection and different dietary regimens on body composition. Mice were fed either a chow or a high-fat diet (HFD) for 12 weeks and 24 weeks. *H. pylori*-infected groups were intragastrically administered either SS1 strain or NCTC11637 strain with a 2 day interval for a total of five infusions. Chow and HFD fed control groups were gavaged with vehicle (Brucella broth). (A) Body weight curves; (B) abdominal circumference; (C) epididymis adipose mass; (D) epididymis adipose mass index (ratio between epididymis adipose mass and body weight); (E) energy intake; (F) energy efficiency (ratio between body weight gain and energy intake). The body weight was significantly increased by four weeks after HFD. $n = 8-10$. Data are expressed as the mean \pm SEM. * $p < 0.05$, ** $p < 0.01$.

homeostasis model assessment of insulin resistance (HOMA-IR) index values further supported that *H. pylori* infection aggravated IR after 12 weeks of HFD consumption (Fig. 2C and E). Moreover, the fasting blood glucose was higher in HFD mice infected with *H. pylori* SS1 than uninfected HFD controls (Fig. 2D), suggesting that *H. pylori* infection had a synergistic effect on the dysregulation of glucose homeostasis. We then measured the expression of insulin signaling proteins in liver and found a substantial reduction in the phosphorylation of IRS1 (Tyr896) and Akt (Ser473) in *H. pylori*-infected mice administered a HFD (Fig. 2F), further confirming the deleterious effects of *H. pylori* infection combined with HFD on IR. However, after long-term HFD consumption, the diet itself had a strong effect on glucose dysregulation that seems greater than *H. pylori* infection, as that the insulin sensitivity was lower and the fasting insulin and HOMA-IR were higher in HFD groups than the chow diet (CD) controls at 24 weeks, with no statistical difference between *H. pylori*-infected and non-infected groups (Fig. 2A, C and E). We also found that the IR of HFD controls increased strikingly at 24 weeks compared with 12 weeks as revealed by IPITTS, whereas the *H. pylori*-infected HFD groups only increased slightly, which supported the crucial role of diet after long-term treatment (Fig. 2G).

3.3. *H. pylori* Infection Influences the Circulating Metabolic Hormones Instead of Inflammatory Cytokines

Chronic inflammation and alterations in metabolic hormones are believed to be responsible for IR. In our study, the serum levels of TNF- α , IL-1 β and IL-6 trended to be higher in animals infected with *H. pylori*

SS1 on a normal diet at 12 weeks, while no difference was observed between *H. pylori*-infected and non-infected groups in HFD (Fig. 3A-C). Similarly, after 24 weeks the levels of these cytokines in HFD-fed *H. pylori*-infected groups were not different from the HFD controls except IL-6 (Supplement Fig. 3A-C). *H. pylori* has been reported to influence physiological regulation of metabolic hormones involved in food intake, energy expenditure and body mass (Khosravi et al., 2015). We found that the serum level of gastric inhibitory polypeptide (GIP) and leptin were higher in animals infected with *H. pylori* when given a normal diet at 12 weeks compared to the non-infected controls, and the level of GIP was further increased in *H. pylori*-infected group fed HFD compared with HFD controls (Fig. 3D and E). Moreover, *H. pylori* infection apparently elevated the circulating level of resistin which was identified to play a significant role in the onset of IR after 12 weeks HFD (Fig. 3F). At 24 weeks the level of resistin increased rapidly in the HFD controls compared with 12 weeks and no significant difference in resistin was observed in HFD groups regardless of *H. pylori* infection status (Fig. 3G and H). These results suggest that *H. pylori* infection influences the secretion of metabolic hormones which associates with the aggravation of IR at an early stage of HFD feeding although the diet itself has a much stronger effect after long-term administration of HFD.

3.4. The Alteration of Gut Microbiota is Correlated with *H. pylori*-associated IR

Accumulating evidence suggests that dysbiosis of gut microbiota plays a vital role in triggering obesity, IR and other metabolic disorders

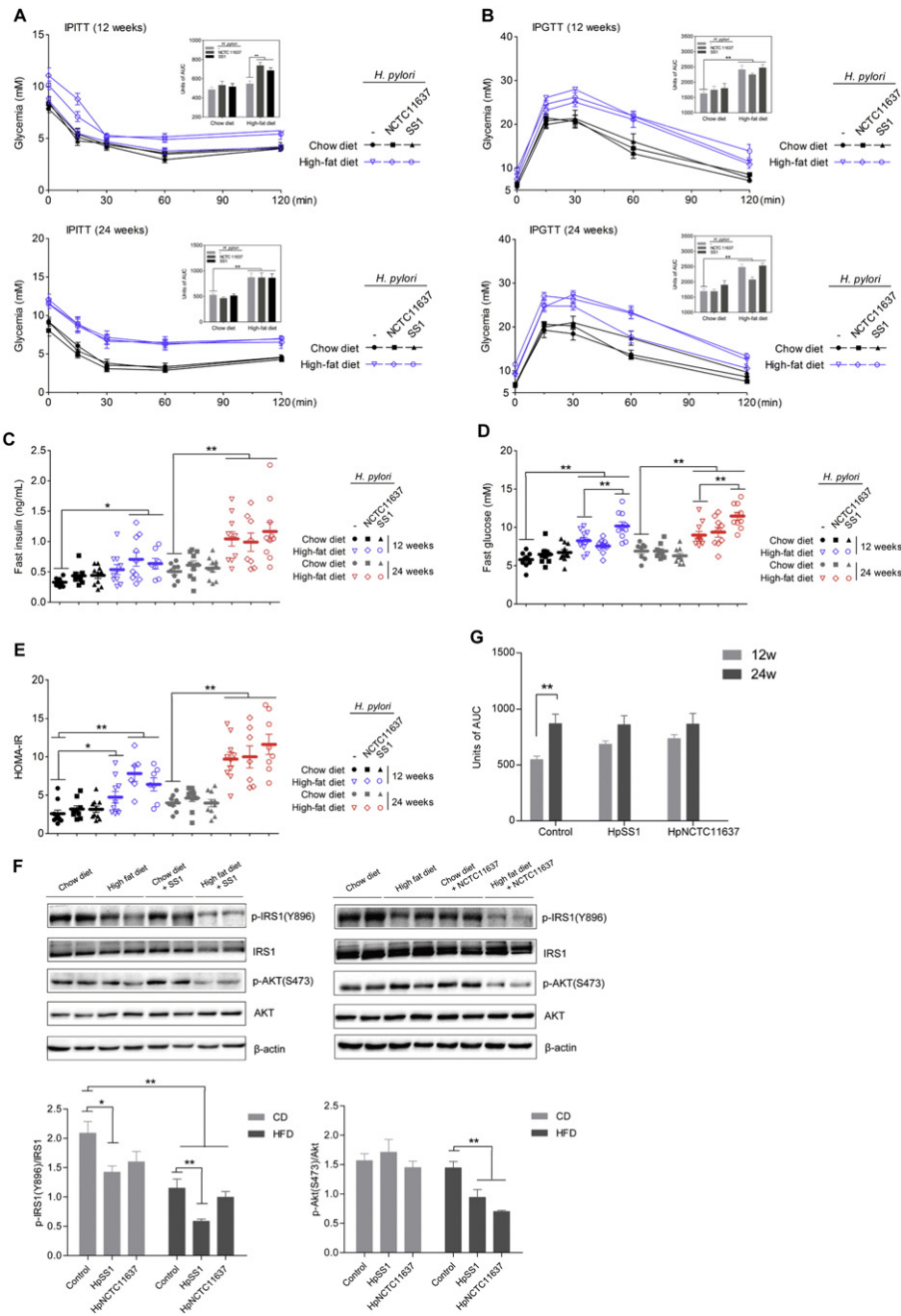


Fig. 2. *H. pylori* infection promotes diet-induced IR and hyperinsulinaemia at the early stage. The intraperitoneal insulin and glucose tolerance tests were performed at different time points during the treatment (A, B). Mice were fasted overnight for plasma insulin (C), blood glucose (D) determination and the HOMA-IR index was calculated (E). Immunoblots of phosphorylated insulin receptor substrate 1 (p-IRS1) (Tyr896) and phosphorylated Akt (p-Akt) (Ser473) in the chow or HFD groups with or without *H. pylori* infection (F). Area under the curve for insulin tolerance tests after 12 and 24 weeks of HFD consumption in *H. pylori*-infected and non-infected groups (G). Data are expressed as the mean \pm SEM. * $p < 0.05$, ** $p < 0.01$.

(Owyang and Wu, 2014). Recent reports have highlighted that there is an ongoing crosstalk between *H. pylori* and the gut microbiota which is associated with the gut inflammation (Khosravi et al., 2015). Thus, we investigated whether the aggravating effect of *H. pylori* on HFD-induced IR is associated with perturbation of the gut microbiota. The 16S rRNA gene sequencing of fecal samples at 12 weeks revealed that *H. pylori*-infected mice feeding HFD had a distinct microbiota community that clustered separately from both the HFD controls and the chow diet groups (Fig. 4A). Compared to the HFD controls at the phylum level, the *H. pylori*-infected group feeding HFD had a significantly increased proportion of sequences assigned to *Firmicutes* and *Proteobacteria*, whereas reads assigned to *Bacteroidetes* and *Verrucomicrobia* were relatively reduced (Fig. 4B). To identify bacterial taxa that were significantly

differentiated between *H. pylori*-infected and non-infected groups feeding HFD, a metagenomic biomarker discovery approach (LEfSe) was used. We found that *Desulfovibrionaceae* and *Mucispirillum* spp. sequences were significantly enriched in the HFD-fed *H. pylori* infected group, as were sequences from the families *Helicobacter*, *Lachnospiraceae*, and *Ruminococcaceae*, while *Akkermansia* spp., *Prevotella* spp., and *Bifidobacterium* spp. were more abundant in HFD controls (Fig. 4C). Given that intestinal dysbiosis in HFD-fed animals may affect gut permeability and subsequently lead to systematic inflammation and IR (Tran et al., 2015), we next examined the expression of the tight junction components including occludin, zonula occludens-1 (ZO-1) and claudin-1 in the intestine. Expression of these proteins was significantly reduced in *H. pylori*-infected

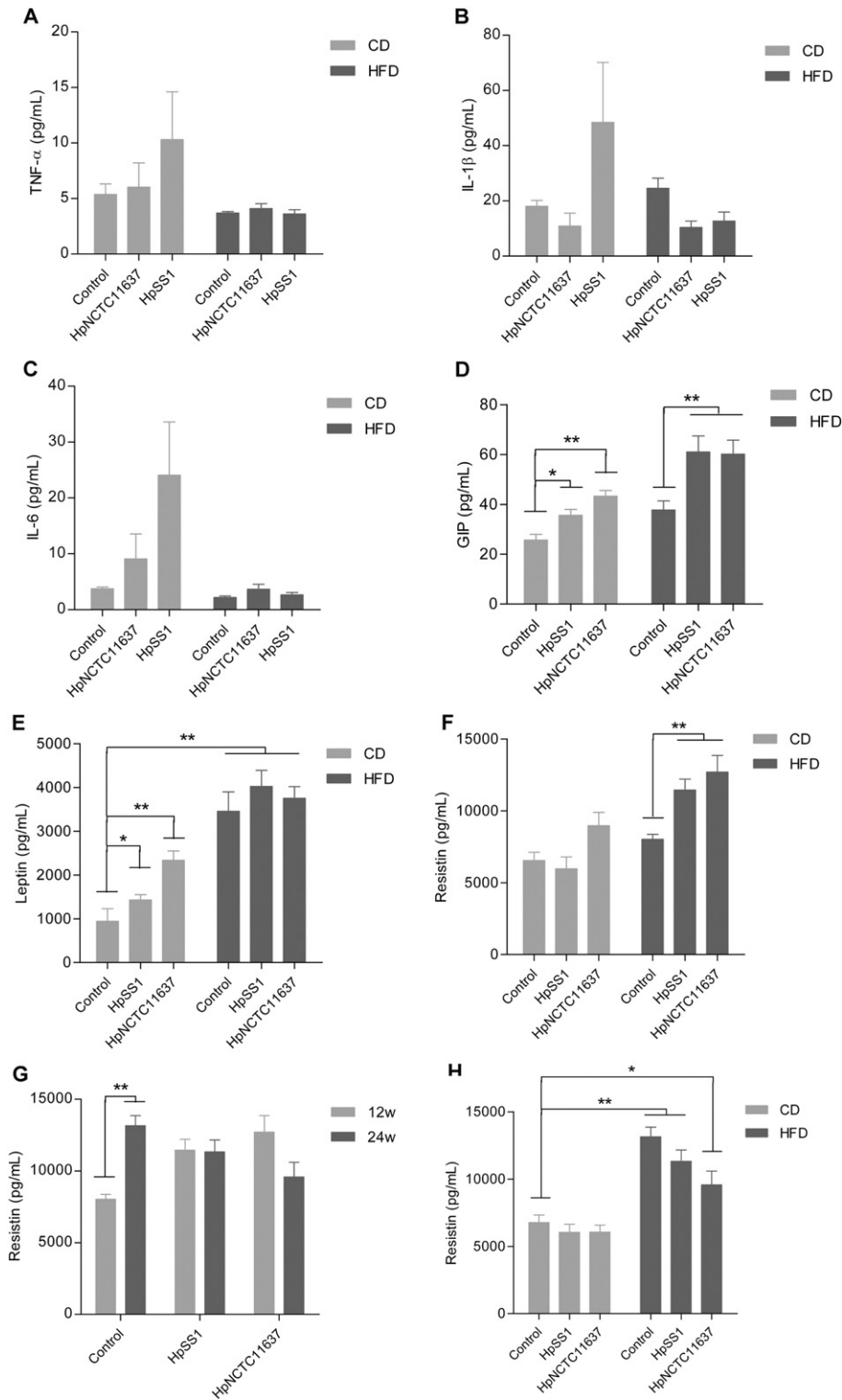


Fig. 3. Effects of *H. pylori* infection with different diets on circulating inflammatory cytokines and metabolic hormones. Serum levels of TNF- α (A), IL-1 β (B) and IL-6 (C) were not significantly different between *H. pylori*-infected and non-infected mice regardless of the diet structure at 12 weeks. Animals with *H. pylori* infection, especially those fed a HFD, showed significant increases in serum levels of metabolic hormones including GIP (D), leptin (E) and resistin (F) compared to their vehicle-treated controls at 12 weeks. The level of resistin increased rapidly in the HFD control group after 24 weeks compared with 12 weeks (G) and thus come up with *H. pylori*-infected groups fed HFD at 24 weeks (H). Data are expressed as the mean \pm SEM. * $p < 0.05$, ** $p < 0.01$.

HFD groups, indicating an aberration of gut barrier function (Fig. 4D). However, at 24 weeks the principal coordinate analysis (PCoA) scores clearly separated the HFD groups from the normal diet groups regardless of *H. pylori* infection status, indicating the dominant role of HFD in modulating gut microbiota after long-term treatment (Fig. 4E and F).

Consistent with the alterations in the gut microbiota, the insulin sensitivity was comparable between *H. pylori*-infected mice and non-infected HFD-fed mice at 24 weeks but was significantly different from the chow diet groups. These results suggest that *H. pylori*-associated IR after early HFD is closely correlated with the specific modifications of gut microbiota.

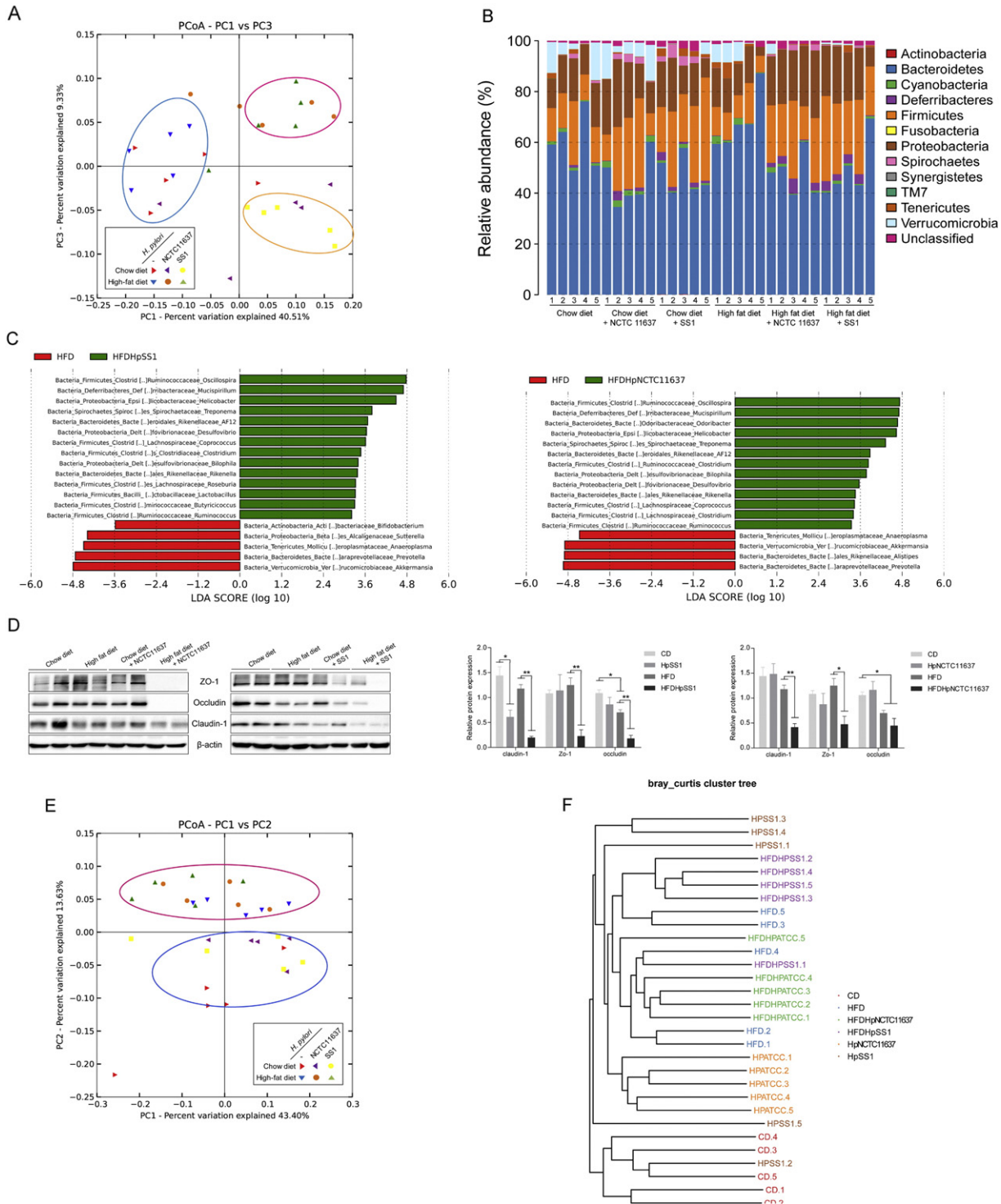


Fig. 4. The interaction of *H. pylori* infection and HFD induces the alteration of gut microbiota which is associated with IR. Microbiota composition was analyzed in faeces of *H. pylori*-infected and non-infected mice fed either chow or HFD (n = 5 for each group). (A) Plots shown were generated using the weighted version of the UniFrac-based Principal coordinate analysis (PCoA) after 12 weeks. Samples from *H. pylori*-infected mice fed HFD included in the red circle clustered distinctly from those of HFD-fed controls embraced in the blue circle. (B) Bacterial taxonomic profiling at the phylum level of gut bacteria from different groups after 12 weeks. (C) Linear discriminant analysis (LDA) coupled with effect size measurements identifies the most differentially abundant taxa between HFD-fed mice with *H. pylori* infection and HFD controls after 12 weeks. HFD controls-enriched taxa are indicated with a negative LDA score (red), and taxa enriched in *H. pylori*-infected HFD group have a positive score (green). Only taxa meeting an LDA significant threshold of >2 are shown. (D) The jejunum tissues of mice were analyzed for the expression of intestinal tight junction proteins including ZO-1, occludin and claudin-1 after 12 weeks. The PCoA (E) and unweighted pair group method with arithmetic mean (UPGMA) (F) analysis showed the alterations of microbial community after 24 weeks. Samples from the HFD group regardless of *H. pylori* infection status clustered together in the red circle which are different from those given chow diet in the blue circle.

4. Discussion

After the first prospective cohort study demonstrated that *H. pylori* infection resulted in an increased rate of incident diabetes, following studies also showed that *H. pylori* infection was significantly associated with higher levels of HbA1c which is a valid and reliable biomarker for long-term blood glucose level in humans (Chen and Blaser, 2012; Hsieh et al., 2013; Jeon et al., 2012). Moreover, a recent randomized double-blind placebo-controlled trial demonstrated that *H. pylori* eradication improves glucose homeostasis in patients with type 2 diabetes by decreasing HOMA-IR and fasting insulin levels (Bonfigli et al., 2016). In a previous study, we also found in Mongolian gerbils that long-term *H. pylori* infection induced increased levels of HbA1c, but its effect on glucose tolerance and the underlying mechanism remains unclear (Yang et al., 2015). Despite the positive association between *H. pylori* and insulin resistance reported in some researches, there are opposite perspectives denying their relationship (Upala and Sanguankeo, 2016). Thus more investigation is needed to determine whether there are some other factors involving in the association between *H. pylori* and insulin resistance which could account for the discrepancies.

Given the increasing intake of high-fat and high-calorie diets in the post-industrialization era and the high prevalence of *H. pylori* infection at a global level, we therefore investigated the impact of *H. pylori* infection in combination with HFD on body insulin sensitivity and subsequent predisposition to metabolic disorders. Our findings of increased visceral fat and larger waist circumference in *H. pylori*-infected mice after 12 weeks HFD, which almost reached the level of 24 weeks of HFD, suggest that *H. pylori* infection may accelerate HFD-induced fat redistribution and the emergence of central obesity. We also demonstrated that *H. pylori* infection aggravated HFD-induced IR, and this effect occurred at the early stage. These results are consistent with previous observation in humans indicating that the role of *H. pylori* in impaired glucose tolerance may be potentiated by higher body mass index level (Chen and Blaser, 2012). In the present study, we showed that the expressions of p-IRS1 (Tyr896) and the downstream protein p-Akt (Ser473) in the liver of mice with *H. pylori* infection were decreased after early HFD administration. The changes in phosphorylation of insulin signaling proteins could directly lead to IR (Xu et al., 2014). After 24 weeks of HFD, we found that the IR of the control group was much higher than that of 12 weeks and almost come up with the *H. pylori*-infected groups. These results suggest that diet itself exerts a strong effect on glucose dysregulation that seems greater than *H. pylori* infection after long-term HFD.

Recently, the gut microbiota has emerged as an essential modulator that influences host metabolism and has been suggested to play a vital role in metabolic diseases. *H. pylori*, an ancient gastric pathogen, has been reported to interact with other gastric microbiota whereas the literature regarding its association with gut microbiota is rare (He et al., 2016). Our study demonstrated that there is an interaction of *H. pylori*, diet and gut microbiota, and the adverse effect of *H. pylori* on IR is associated with gut microbiota. Using 16S rRNA gene sequence analysis, we showed that *H. pylori* induced a dramatic shift in the gut microbiota of mice feeding HFD by increasing the proportion of *Firmicutes* and decreasing the proportion of *Bacteroidetes*. This phylum-wide pattern of compositional reshape in the microbial community has been thought to be a typical characteristic of obesity-driven dysbiosis as shown in both overweight humans and animals (Cotillard et al., 2013; Mozes et al., 2008). Additionally, *H. pylori* infection with HFD induced a decreased percentage of *Verrucomicrobia*, which was mostly contributed by a significant reduction of *Akkermansia* spp. *Akkermansia* within the mucus layer has been implicated in regulating the gut barrier function and protecting the host from obesity-linked metabolic syndrome (Everard et al., 2013; Roopchand et al., 2015). Oral administration of this bacterium (i.e., as a probiotic) has been reported to reverse HFD-induced metabolic disorders and also mimic the antidiabetic

effects of metformin in diabetic mice (Everard et al., 2013; Shin et al., 2014). In contrast, several bacterial families like *Desulfovibrionaceae*, *Ruminococcaceae* and *Lachnospiraceae* were enriched, which is consistent with prior reports that similar alterations of the microbial communities are positively correlated with the development of metabolic diseases (Kameyama and Itoh, 2014; Kim et al., 2012; Zhang et al., 2010). Identified as the potentially important endotoxin producers, members in *Desulfovibrionaceae* are capable of reducing sulphate to H₂S and damaging the gut barrier (Zhang et al., 2010). Our results showed that *H. pylori* infection in combination with HFD decreased the expression of tight junction proteins in the intestine, and the impairment of intestinal integrity may in turn facilitate the release of endotoxin into the bloodstream and therefore promote metabolic inflammation and IR (Cani et al., 2008). After 24 weeks, the microbiota community from HFD-fed mice with *H. pylori* infection clustered together with the HFD controls, both of which were separated from the normal diet groups. Thus, we speculate that there is a close correlation between gut microbiota and metabolic phenotype and changes in several key microbiota populations after *H. pylori* infection play a critical role in the aggravation of IR.

Although the serum levels of some inflammatory cytokines such as TNF- α were not elevated in *H. pylori*-infected HFD mice, the increased abundance of *Desulfovibrionaceae* which can produce endotoxins and the compromised intestinal barrier cast some doubt on the role of inflammation. In addition to inflammation hypothesis, increasing evidence indicates that *H. pylori* infection can influence the production of metabolic hormones involved in energy homeostasis both in human and animal studies, which is another potential mechanism for *H. pylori*-associated insulin resistance (Boltin and Niv, 2012; Khosravi et al., 2015; Roper et al., 2008). In our study, we also found remarkable changes of metabolic hormones after *H. pylori* infection given HFD. Both GIP and resistin which have been reported to play a cardinal role in IR and associated metabolic disturbances were observed to increase significantly in *H. pylori*-infected mice with HFD at the early stage (Gault et al., 2005; Muse et al., 2004). Moreover, the HFD itself induced dramatic elevation of circulating resistin after long-term treatment, and thus no statistical difference was observed between *H. pylori*-infected and non-infected groups. The changes of resistin are consistent with the alteration in body IR, which indicates the role of metabolic hormones in *H. pylori*-associated IR.

Both *H. pylori* strains used in this study are observed to promote HFD-induced metabolic disorders, although the degree of their pathogenicity is not identical. The discrepancies in the association of *H. pylori* and IR in epidemiological studies are likely due to the infection of different strains as well as diet consumption. Further experiments using germ-free animals are highly warranted to elucidate the causal relationship between gut microbiota and *H. pylori*-associated IR in the presence of HFD.

In conclusion, we found that *H. pylori* infection promoted HFD-induced central obesity and IR at the early stage, the extent of which is close to the effect of long-term HFD. Meanwhile, we observed a dynamic alteration in the gut microbiota that is consistent with the changes of metabolic phenotype (Supplement Fig. 4). Thus, there may be an interaction among *H. pylori*, diet and gut microbiota, which dysregulates host metabolic homeostasis and the treatment of *H. pylori* may be beneficial to the patients with impaired glucose tolerance in addition to diet control.

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.ebiom.2016.09.010>.

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Conflicts of Interest

No author had any financial or personal relationships that could inappropriately influence or bias this work.

Author Contributions

Cong He and Zhen Yang were involved in all aspects of the study. Dandan Cheng, Chuan Xie and Yin Zhu were involved in acquisition, analysis and interpretation of data. Zhongming Ge and Zhijun Luo were involved in revision of the manuscript. Nonghua Lu was involved in study concept, design and supervision.

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