Sex Differences in Gut Microbiome in High-Fat-Diet Fed Rats

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Research

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Abstract

Background: Sex differences in obesity and related metabolic diseases are well recognized, however, the mechanism has not been elucidated. Gut microbiota and its metabolites may play a vital role in the development of obesity and metabolic diseases. The aim of the present study was to investigate sex differences in gut microbiota and its metabolites in a high-fat-diet (HFD) obesity rats and identify microbiota genera potentially contributing to such differences in obesity and non-alcoholic fatty liver disease (NAFLD) susceptibility.

Methods: Sprague–Dawley rats were divided into four groups (eight animals per group): (1) male rats on a normal diet (MND), (2) male rats on HFD (MHFD), (3) female rats on a normal diet (FND), and (4) female rats on HFD (FHFD). Body weight, liver pathology, gut microbiota and short/medium chain fatty acids in colon contents were compared between different sexes.

Results: HFD induced more body weight gain and fat storage in female rats, however, lower hepatic steatosis in FHFD than in MHFD rats was observed. When considering gut microbiota composition, FHFD rats had lower microbiome diversity than MHFD. A significant increase of *Firmicutes* phylum, along with *Bilophila* and *Blautia* genus was detected in MHFD rats, as compared with FHFD, which showed increased relative abundance of *Murimonas*. Moreover, propionic and lauric acid levels were higher in FHFD than those in MHFD rats.

Conclusions: HFD induced sex-related alterations in gut microbiome and fatty acids. Furthermore, the genus *Bilophila*, *Blautia* and *Murimonas* might contribute to sex differences observed in obesity and NAFLD susceptibility.

Background

Nowadays, growing epidemic of obesity represents a great challenge to public health, which is associated with various metabolic disorders, including hyperlipidemia, hypertension, cardiovascular diseases, type 2 diabetes, and non-alcoholic fatty liver disease (NAFLD)[1]. However, men and women exhibit significant differences in obesity and related metabolic diseases. In most countries, obesity prevalence in women is higher than that in men. According to the World Health Organization, in 2016, 11% of men and 15% of women were obese[2]. In addition, a gender difference in body fat distribution is recognized, although men have lower percentage of total body fat than women, they are more susceptible to abdominal adiposity, whereas women accumulate more adipose tissue in thighs and hips[3]. Furthermore, prevalence of related metabolic diseases, such as type 2 diabetes and NAFLD, is higher in men than that in women[4]. To date, the exact mechanism of these observed sex difference has not been elucidated.

As a hidden metabolic organ, gut microbiota has recently emerged as an important modulator in the development of obesity and related diseases[5]. In fact, there is growing evidence showing gut microbiota involvement in energy regulation, nutrient absorption, and fat storage[6]. Germ-free mice have reduced

adiposity when compared with colonized counterparts, which protects them from high-fat-diet (HFD)-induced obesity[7,8]. In addition, microbiota from an obese mouse may promote weight gain in a germ-free recipient mouse that confirmed the link between host metabolism and gut microbiota[9].

Gender is an important factor altering gut microbiota. In this regard, several studies have provided evidence for gender differences in microbiota composition[10]. For instance, in a research using non-obese diabetic mice, males were observed to bear higher abundance of *Porphyromonadaceae*, *Veillonellaceae*, and *Kineosporiaceae* families than that of females[11]. In addition, a large Chinese cohort study demonstrated that BMI-associated differences in gut microbiota composition was influenced by gender[12]. HFD is commonly used to establish a model of obesity and metabolic syndrome in rodents, and prolonged HFD in rats model mimics human obesity and obesity-related metabolic diseases[13]. However, the role of sex specific changes of gut microbiota in response to HFD-induced-obesity has not been yet demonstrated.

In the present study, we treated male and female rats with HFD for 16 weeks, and analyzed gut microbiota composition and metabolites (short-chain fatty acids (SCFAs) and medium-chain fatty acids (MCFAs)) in colonic contents. The aims of this study were to investigate sex differences in gut microbiota and its metabolites in an HFD obesity rat model and determine microbiota genera potentially contributing to such differences in obesity and NAFLD susceptibility.

Methods

Animals.

Six-week-old male and female specific-pathogen-free (SPF) Sprague–Dawley rats (n = 16 for each gender), weighing 227 ± 28 g, were purchased from the Hunan SJA Laboratory Animal Co., Ltd. (Hunan, China). After one week of acclimatization, rats were randomly divided into the following 4 groups (n = 8 each): (1) male rats on a normal diet (MND), (2) male rats on a high-fat-diet (MHFD), (3) female rats on a normal diet (FND), and (4) female rats on a high-fat-diet (FHFD). All rats were housed by gender placing two individuals per cage in a SPF animal facility, under constant conditions (temperature, 22 ± 1 ℃; humidity, 55% ± 5%; 12 hours dark-light cycle) at the laboratory animal center of Xiangya Medical School of Central South University (Changsha, China) and given free access to standard rat chow (ND: 12% fat, 66% carbohydrate, and 22% protein; 3.5 kcal/g) or HFD (37% fat, 17.5% protein, and 45.5% carbohydrate; 4.50 kcal/g) (purchased from Medical Animal Center of Guangdong Province) and sterile drinking water. Body weight and food intake were weekly monitored throughout the study. After 16 weeks of treatments, rats were anesthetized with 40 mg/kg of sodium pentobarbital by intraperitoneal injection and colonic contents and liver tissues were collected, immediately frozen in liquid nitrogen, and kept at -80℃, until use. All rats used for tissue collection were euthanized by overdose intraperitoneal pentobarbital[14]. This study was conducted according to the Guide for the Care and Use of Laboratory Animals of Central South University and approved by the Institutional Animal Care and Use Committee of Xiangya Medical College.

Dual-energy X-ray Absorptiometry (dxa)

All rats were placed in a prone position and scanned by DXA using the Hologic ultra-high-resolution rat whole body composition software, which measures trunk fat ratio and visceral fat ratio.

Histological Examination

Paraffin-embedded liver sections were stained with hematoxylin and eosin (Sigma-Aldrich, St. Louis, MO, USA) to evaluate status of liver damage using the NAFLD Activity Score (NAS) including steatosis, lobular inflammatory, and ballooning[15]. All histological assessments were performed by a pathologist who was blinded to the treatment.

Gut microbiota analysis.

Bacterial DNA was extracted from colonic contents with the QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany), according to manufacturer's instructions. Extracted DNA was evaluated using 1% agarose gel. Bacterial genomic DNA was used as the template for amplification of 16S rRNA gene V3-V4 hypervariable region with the following primers: primer F, Illumina adapter sequence 1 + CCTACGGGNGGCWGCAG and primer R, Illumina adapter sequence 2 + GACTACHVGGGTATCTAATCC, and specific sequencing labels were added to the library. PCR products were purified and concentrations were adjusted for sequencing on an Illumina Miseq platform with the 2 × 250 bp paired-end method. Raw data were filtered by several steps to remove low-quality reads. Operational taxonomic units (OTUs) were clustered based on a similarity threshold of 97% using UPARSE, and OTUs were randomly subsampled. Good's coverage > of 99.8% for all sequences in the four groups. R software packages (V2.15.3) were employed for calculation of alpha and beta diversities. Microbial community differences of groups were revealed by linear discriminative analysis (LDA) effect size (LEfSe) tests[16]. Gut microbiota functional profiles were predicted using the PICRUSt software package. PICRUSt predicts the metagenome in fecal DNA samples based on the 16S rRNA database and KEGG orthologs[17].

Measurement Of Scfas And Mcfas

Colon contents were stored at -80 ℃ until analysis. SCFAs and MCFAs in colon contents were measured using HPLC-MS/MS method as previously described[18]. Fatty acids were extracted from samples using solvent mixtures containing acetonitrile and water. Butyric-2,2-d2 and 13C1-octanoic acid were used as internal standards for quantitation. Extract was then analyzed by an Exion UPLC system coupled with a mass spectrometer (6500 Plus Qtrap; SCIEX). The instrumental operating parameters of electrospray ionization model were the following: curtain gas = 20, ion spray voltage = 5500 V, temperature = 400 ℃, ion source gas 1 = 35, and ion source gas 2 = 5.

Statistical analysis.

Values were expressed as mean ± SEM. Statistical analyses were performed by IBM SPSS Statistics 22.0 (IBM Corp., Armonk, NY, USA). To determine the statistical differences between two groups, we used the Student´s t test and the Mann–Whitney test. Data sets involving more than two groups were assessed by one-way analysis of variance (ANOVA), followed by Bonferroni's post hoc test or by the non-parametric Kruskal-Wallis test with Dunn's multiple comparisons test. Correlations were determined with Spearman's correlation test.

Results

Sex differences in HFD-induced obesity phenotypes

HFD feeding increased energy intake in male and female rats, but females had lower caloric intake than their diet-matched male counterparts (Fig. 1a). As shown in Fig. 1b, body weights of male and female rats on HFD were higher ($p < 0.05$) than those of ND rats at all subsequent time points after 3 weeks. At the end of the diet period, MHFD and FHFD rats gained 21.47% and 30.26% body weight as compared with MND and FND, respectively (Fig. 1c). No significant differences in weight gain efficiency were observed between MHFD and MND, whereas HFD increased weight gain efficiency in female rats, which correlated with their body weight gain percentage induced by HFD (Fig. 1d). Moreover, upon exposure to HFD, both male and female rats gained significantly more trunk fat and visceral fat ratio compared to ND groups. (Fig. 1e, f).

MHFD rats developed liver steatosis with inflammation, whereas marginal steatosis and inflammatory infiltration were observed in livers of FHFD rats. Similarly, NAFLD activity score (NAS) was significantly lower in FHFD than in MHFD rats (Fig. 1g, h).

Sex Differences In Gut Microbiota Overall Structure

Among the 1,474 operational taxonomic units (OTUs) detected across samples, 632 and 693 OTUs discriminated between MHFD and MND, and FHFD and FND rats respectively (Fig. 2a). The observed species and taxon diversity (Shannon index) of HFD groups were significantly reduced related to control levels (Fig. 2b, c). No significant difference in species richness was observed between MHFD and FHFD rats, whereas the FHFD group had even lower microbiome diversity than that of MHFD. Furthermore, Principal Co-ordinates Analysis (PCoA) revealed a difference in gut bacterial assortment between HFD groups and their respective controls in both sexes and showed clear separations between MHFD and FHFD along the axis2, whereas MND and FND groups were close to each other (Fig. 2d).

Sex-dependent microbial composition changes in HFD rats.

At the phylum level, a significant effect of HFD-feeding was observed in male and female rats (Fig. 3a). A significant increase of *Firmicutes* was detected in MHFD grouop *vs* MND group, but relative low abundance of *Firmicutes* was found in FHFD *vs* FND rats (Fig. 3b). *Bacteroidetes* were found significantly

less abundant in MHFD as compared with controls, and a similar trend was observed in females (Fig. 3c). Notably, despite contrasting changes of *Firmicutes*, the ratio of *Firmicutes* to *Bacteroidetes* was increased in MHFD (13.9 *vs* 2.1) and FHFD (20.3 *vs* 13.1). Furthermore, FHFD rats had a significantly increased level of *Verrucomicrobia*, as compared with that of the MHFD group (Fig. 3d).

Bacterial taxa differences among different groups.

Linear discriminant analysis effect size (LEfSe) algorithm was applied to identify key phylotypes responsible for differences between MND and FND, so did MHFD and FHFD (Fig. 4a, c). A cladogram of significantly different taxa in order and family level abundance is shown in Fig. 4b and Fig. 4d, and detailed results at genus level are presented in Table 1. Among key genera with a relative abundance > 1% in at least one group, *Bacteroides* and *Murimonas* were significantly more abundant in the MND, relative to levels in female rats. Meanwhile, high levels of *Acetanaerobacterium, Bacteroides, Bilophila, Blautia* and *Romboutsia* were found in MHFD, whereas the FHFD group possessed obviously increased proportions of *Akkermansia* and *Murimonas*.

Table 1
Sex differences in relative abundance of key genera in HFD rats

	MND	FND	P value; Fold change	MHFD	FHFD	P value; Fold change
Acetanaerobacterium	0.004 ± 0.004	0.048 ± 0.047	P > 0.05; 0.08	1.539 ± 0.263	0.575 ± 0.065	0.039; 2.68
Akkermansia	0.269 ± 0.209	8.966 ± 5.654	P > 0.05; 0.03	7.010 ± 2.864	43.894 ± 3.828	P < 0.01; 0.16
Bacteroides	2.168 ± 0.454	0.451 ± 0.112	0.043; 4.78	3.252 ± 0.737	0.567 ± 0.359	0.042;5.74
Bilophila	0.088 ± 0.019	0.166 ± 0.059	P > 0.05; 0.53	2.983 ± 0.809	0.127 ± 0.058	0.046;23.49
Blautia	0.945 ± 0.433	1.172 ± 1.143	P > 0.05; 0.81	14.853 ± 2.074	6.629 ± 1.061	0.028; 2.24
Murimonas	0.498 ± 0.111	0.061 ± 0.033	0.036; 8.16	0.332 ± 0.053	1.052 ± 0.196	0.048; 0.32
Romboutsia	11.422 ± 1.400	8.824 ± 2.070	P > 0.05; 1.29	13.160 ± 1.792	6.737 ± 1.371	0.013; 1.95

Key genera were identified by LEfSe analysis and only genera with an LDA significant threshold > 2 and relative abundance > 1% in at least one group are shown. Data are expressed as mean ± SEM. Statistical significances were determined by one-way ANOVA.

Sex-dependent metabolic changes in HFD rats.

HFD groups had a significant increase in propionic acid and decreases in isobutyric, butyric, and valeric acid levels in males and females, however, FHFD had even higher propionic acid than MHFD, as compared with controls. In addition, a significant increase in acetic acid was only identified in MHFD, as compared with controls (Fig. 5a).

For MCFAs, HFD feeding was associated with reduced heptanoic acid level and elevated nonanoic acid amounts in both sexes. Moreover, iso-heptanoic acid level was significantly increased in male rats, but decreased in females after HFD, as compared with control group, whereas undecanoic and lauric acid levels only increased in FHFD rats (Fig. 5b).

Gut Microbiota Functional Analysis

Overall, HFD had a significant effect on the predicted metabolic pathways in male and female rats, based on phylogenetic investigation of communities by reconstruction of unobserved states (PICRUSt) analysis (Fig. 6); 162 pathways were altered between MHFD and MND groups, whereas 155 were altered between FHFD and FND groups. Metabolism-related pathways, such as arginine and proline, glycerophospholipid, and pyruvate metabolism, and pentose phosphate pathway were upregulated in HFD groups in both sexes. However, mannose, galactose, glycerolipid, and starch metabolism, as well as fructose pathway were only enriched in the MHFD group. Similarly, folate biosynthesis, and glycine, serine, and threonine metabolism were more abundant in the FHFD group.

Correlations between key genera and phenotype in HFD rats.

The strong relationship between the relative abundance of key genera and phenotypes of different genders were shown in Fig. 7. In particular, the increase of *Blautia* and decrease of *Clostridium cluster IV*, *Barnesiella*, and *Alistipes* were associated with weight gain, and trunk and visceral fat ratio in male rats, whereas the increased level of *Akkermansia*, *Acetanaerobacterium*, and *Murimonas* and reduced level of *Alloprevotella*, *Paraprevotella*, and *Eisenbergiella* were observed to have substantial relationships with weight gain and fat ratio in females. NAS score was positively correlated with *Blautia*, *Bilophila*, *Anaerovorax*, and *Collinsella* in males, and positively related with *Murimonas* and *Roseburia* in female rats. Some genera had positive relationships with propionic acid levels in male rats, including *Bilophila*, *Anaerovorax*, and *Collinsella*, whereas *Murimonas* positively correlated with propionic acid in females.

Discussion

Results on sex differences in response to HFD are controversial. Some reported female HFD-fed rats gained more weight and had increased adiposity index than male HFD-fed rats[19], but some found the opposite[20]. Such a discrepancy could be due to differences in diet composition or diet period. In this 16-week study, we found female HFD-fed rats gained more body weight than male HFD-fed rats. Weight gain efficiency is the mark of metabolic control of obesity, and it represents the energy that saved as extra body energy stores. Our results showed that weight gain efficiency was higher in FHFD than MHFD. The

higher weight gain efficiency in FHFD may contribute to more body weight gain percentage induced by HFD in females. Also, we found that FHFD rats had less severity of hepatic steatosis and inflammation than MHFD rats, which agrees with previous studies[21].

Traditionally, sex differences in susceptibility of obesity and metabolic diseases have been explained by the different effects of sex hormones, which influence many aspects of metabolism and related diseases[22]. Nowadays, gut dysbiosis has been suggested to play a crucial role in mediating the interaction between consumption of HFD and development of obesity in host. Whether there are sex specific changes of gut microbiota in response to HFD, as well as its role in metabolic diseases remains to be explored.

In the present study, we found that HFD feeding decreased the taxon diversity of gut microbiota in male and female rats, but FHFD had even lower microbiome diversity than that of MHFD. It has been confirmed that obesity is associated with a reduced diversity of microbiota in rodents and humans[23,24]. Therefore, lower microbiome diversity in FHFD may partly be attributed to the higher body weight gain percentage in females on HFD.

We used LEfSe analysis to identify sex-specific characteristic key taxa. At the genus level, we found that *Bilophila* abundance was significantly increased only in the male rats after HFD-fed, and it positively corelated with weight gain and NAS score in male rats. *Bilophila* is a lipopolysaccharide-producing bacterium, previously reported to increase in response to HFD[25,26]. *Bilophila wadworthia* was found to synergize with HFD to promote intestinal barrier dysfunction and bile acid dysmetabolism, leading to hepatic steatosis[27]. In the FHFD group, we did not observe higher *Bilophila* abundance, which may suggest that changes in *Bilophila* abundance contributes to the gender differences in NAFLD severity.

In our study, the HFD groups exhibited an increase in the abundance of *Blautia*, and this change was more apparent in MHFD rats. A positive correlationship between abundance of *Blautia* and obesity has been reported in Chinese and American youths[28,29]. Obesity is a well-known risk factor for the development of NAFLD. Shen et al.[30] found an enrichment of *Blautia* in NAFLD patients, and considered it as a primary contributor to NAFLD progression. Thus, we hypothesized that the high abundance of *Blautia* in the MHFD group maybe contribute to the more severity of hepatic steatosis in male rats.

Notably, a significant increase of *Murimonas* was only detected in the FHFD group, which was positively correlated with weight gain and fat ratio in female rats, but not in males. Few reports about the relationship between *Murimonas* and HFD were found till now, since most of the studies were conducted in male rats. The strong correlationship between *Murimonas* and weight gains in FHFD group may suggest a key role of *Murimonas* in obesity in female subjects.

Akkermansia muciniphila is a mucin-degrading bacterium which involved in maintaining intestinal integrity[31]. And a number of studies have claimed the probiotic effects of *A.muciniphila* on obesity and metabolic disorders[32]. However, in our study, the relative abundance of genus *Akkermansia* was higher in

rats fed with HFD in both sexes. First, it's important to note that *A.muciniphila* is the only species that has been identified in this genus so far, and the characteristics of other species of genus *Akkermansia* need to be further confirmed. Then, there were also a few other studies found high levels of *A.muciniphila* in HFD-fed-rats[33], suggesting the need for caution in claiming the beneficial effect of *A. muciniphila*.

We also investigated sex-related SCFAs and MCFAs alterations after HFD feeding. HFD groups were shown to significantly increase propionic acid, particularly, FHFD had higher propionic acid than MHFD. In fact, the role of propionic acid in obesity development is still controversial. In this regard, a previous study showed that propionic acid may have beneficial effect on visceral adipose tissue by reducing obesity associated inflammation[34]. Furthermore, propionic acid was reported to reduce lipogenic enzymes expression in liver, resulting in decreased hepatic triglycerides and prevention of NAFLD by suppressing fatty acid synthesis and oxidative stress[35]. On the other hand, fecal SCFAs concentration in obese volunteers was higher compared with that of lean individuals in human studies, since they provided additional energy to the host[36], and NAFLD patients had higher fecal propionate levels, which was positively associated with lower resting regulatory T-cells in peripheral blood, as immunological characteristics of NAFLD patients[37]. We considered that the high concentration of propionic acid in FHFD might partly contribute to the alleviation of liver steatosis in female rats by reducing hepatic lipogenesis and fatty acid uptake. For MCFAs, the level of lauric acid was elevated only in female rats after HFD. Lauric acid was a medium-chain saturated fatty acid and a recent study showed that lauric acid alleviated obesity in HFD-fed male rats and prevented hepatic lipid accumulation[38,39]. However, the effects of lauric acid on fat storage and hepatic steatosis in female rats is still elusive.

To address the role of gut microbiota alterations in metabolic pathways, HFD-induced functional alterations were predicted by PICRUSt. Pathways related to carbohydrate metabolism were enriched in the MHFD group, whereas parts of amino acid metabolism pathways were more evident in FHFD. It may suggest that HFD feeding modifies the functional metagenome in a sex-specific way.

The present study had some limitations, including the lack of measuring fat ratio during the feeding period, because of which we did not observe the fat ratio dynamic trend of different genders. Another constraint was that we only detected SCFAs and MCFAs in the colonic content, without measuring other gut microbiota metabolites. Finally, since NAFLD occurs in liver-specific androgen receptor knockout mice[40], it cannot be elucidated that the sex differences seen in our study are definitely mediated by intestinal bacteria. Gut microbiota transplantation needs to be performed to verify the causal relationship.

Conclusions

Our results revealed that HFD feeding exhibited sex-dependent effects on gut microbiome and host metabolism in Sprague-Dawley rats. In addition, we found three main genera, *Bilophila*, *Blautia* and *Murimonas*, which may potentially contribute to the gender differences in body weight gain and liver steatosis. More attention should be given to gut microbiota composition to explain the sex differences of diet-induced obesity and hepatic steatosis.

Perspectives And Significance

In summary, our results may help to explain gender disparities in obesity from the aspect of gut microbiota and metabolites, which have become the new frontiers in understanding metabolic diseases. Future works should make causal inferences about the role of the main gut microbiota genera and provide possible evidence for the treatment of metabolic diseases by ameliorating intestinal flora disturbance, leading to a accurate and individualized treatment for obesity patients.

Abbreviations

HFD
high-fat-diet; NAFLD:non-alcoholic fatty liver disease; SCFAs:short-chain fatty acids; MCFAs:medium-chain fatty acid; NAS:NAFLD activity score; OTUs:operational taxonomic units; PCoA:Principal Co-ordinates Analysis; LEfSe:linear discriminant analysis effect size; PICRUSt:phylogenetic investigation of communities by reconstruction of unobserved states; SPF:specific-pathogen-free; DXA:Dual-energy X-ray absorptiometry; LDA:linear discriminative analysis; ANOVA:analysis of variance

Declarations

Ethics approval

The study was conducted according to the Guide for the Care and Use of Laboratory Animals of Central South University and approved by the Institutional Animal Care and Use Committee of Xiangya Medical College (NO: 2018sydw184).

Consent for publication

Not applicable.

Availability of data and materials

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

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

DZ and KX supervised and designed the study, analyzed the data and wrote the paper. FY, LW, LX, SW and XX performed the animal experiment. GS, RR and SML conducted the SCFAs and MCFAs measurements and contributed to the preparation of all the figures. YS performed the animal experiment, analyzed the data and wrote the paper. All authors reviewed the results and approved the final version of the manuscript.

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Figures

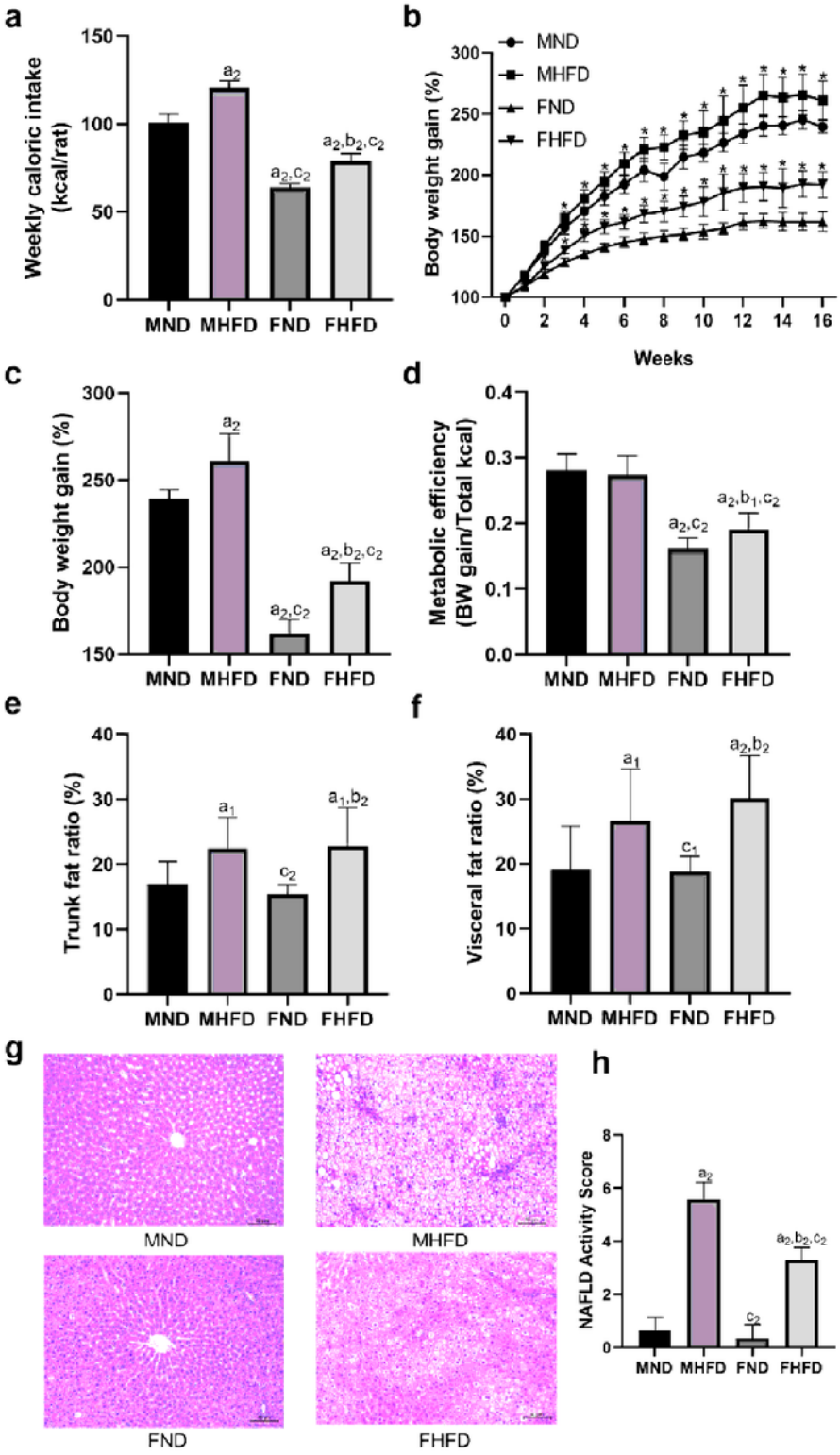

Figure 1

Sex differences in obesity and NAFLD in HFD-fed rats. (a) Weekly caloric intake of rats. (b) Body weight gained percentage during 16 weeks of ND or HFD feeding in rats. (c) Weight gain percentage at the end of experiment. (d) weight gain efficiency (body weight gain divided by the total energy intake). (e) Trunk fat ratio of body weight. (f) Visceral fat ratio of body weight. (g) Representative pictures of H&E staining in liver tissue (200×). (h) NAFLD activity Score. Data are expressed as mean ± SEM. (a, c, d, e, f, h) a1p<0.05,

a2p<0.01 vs MND group; b1p<0.05, b2p<0.01 vs FND group; c1p<0.05, c2p<0.01 vs MHFD group. (b) *p<0.05 when compared to ND group of same sex. (a-f) Statistical significances were determined by one-way ANOVA. (g) Statistical significances were determined by non-parametric test.

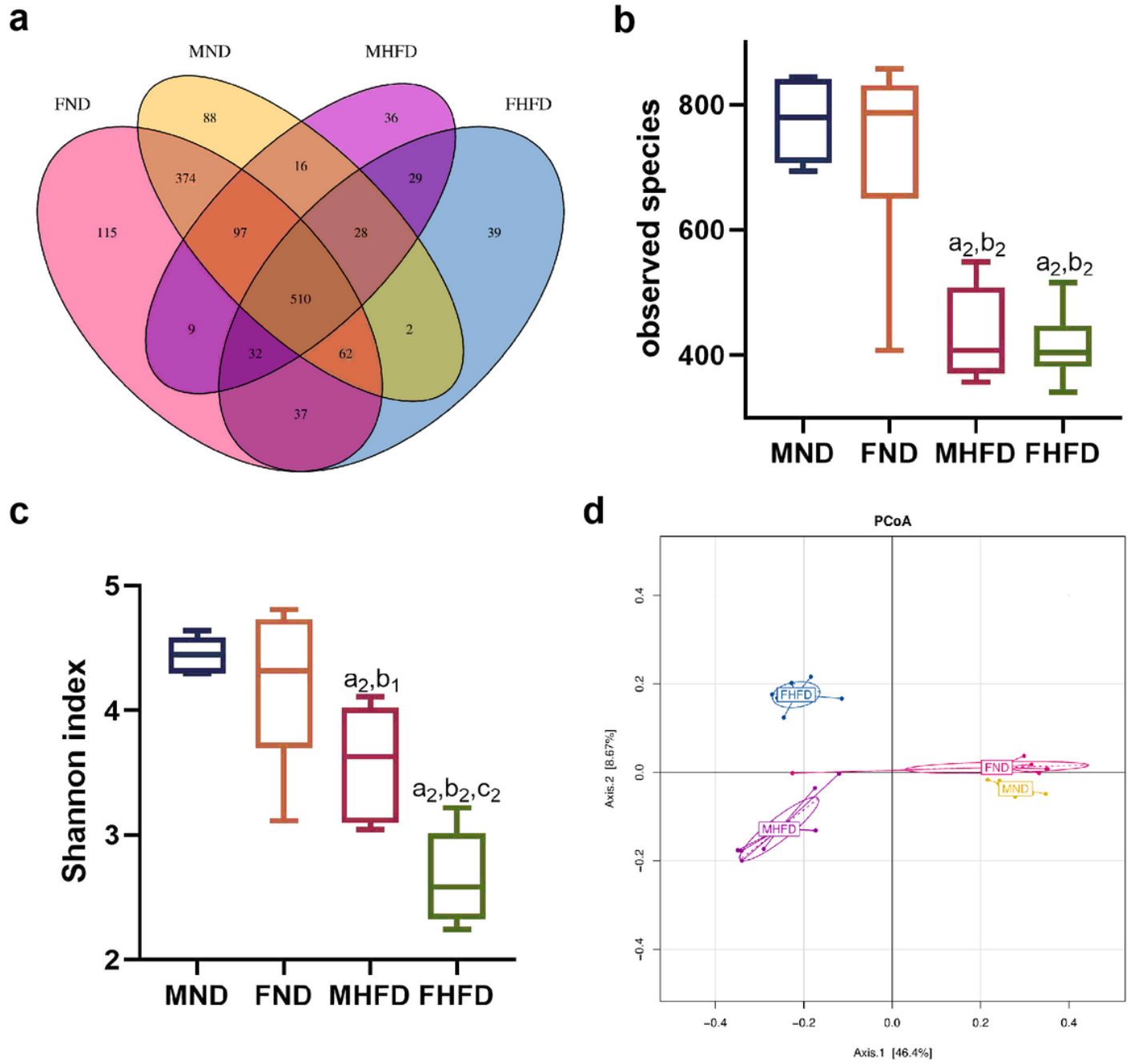

Figure 2

Alpha and Beta-diversity in ND or HFD-fed rats of both genders. (a) Venn diagram representation of shared/unique OTUs in the fecal microbiota among groups. (b) Observed species. (c) Shannon index. (d) PCoA analysis based on jaccard distance. Data are expressed as mean ± SEM. a1p<0.05, a2p<0.01 vs MND group; b1p<0.05, b2p<0.01 vs FND group; c1p<0.05, c2p<0.01 vs MHFD group. (b, c) Statistical significances were determined by one-way ANOVA. (d) Statistical significances were determined by non-parametric test.

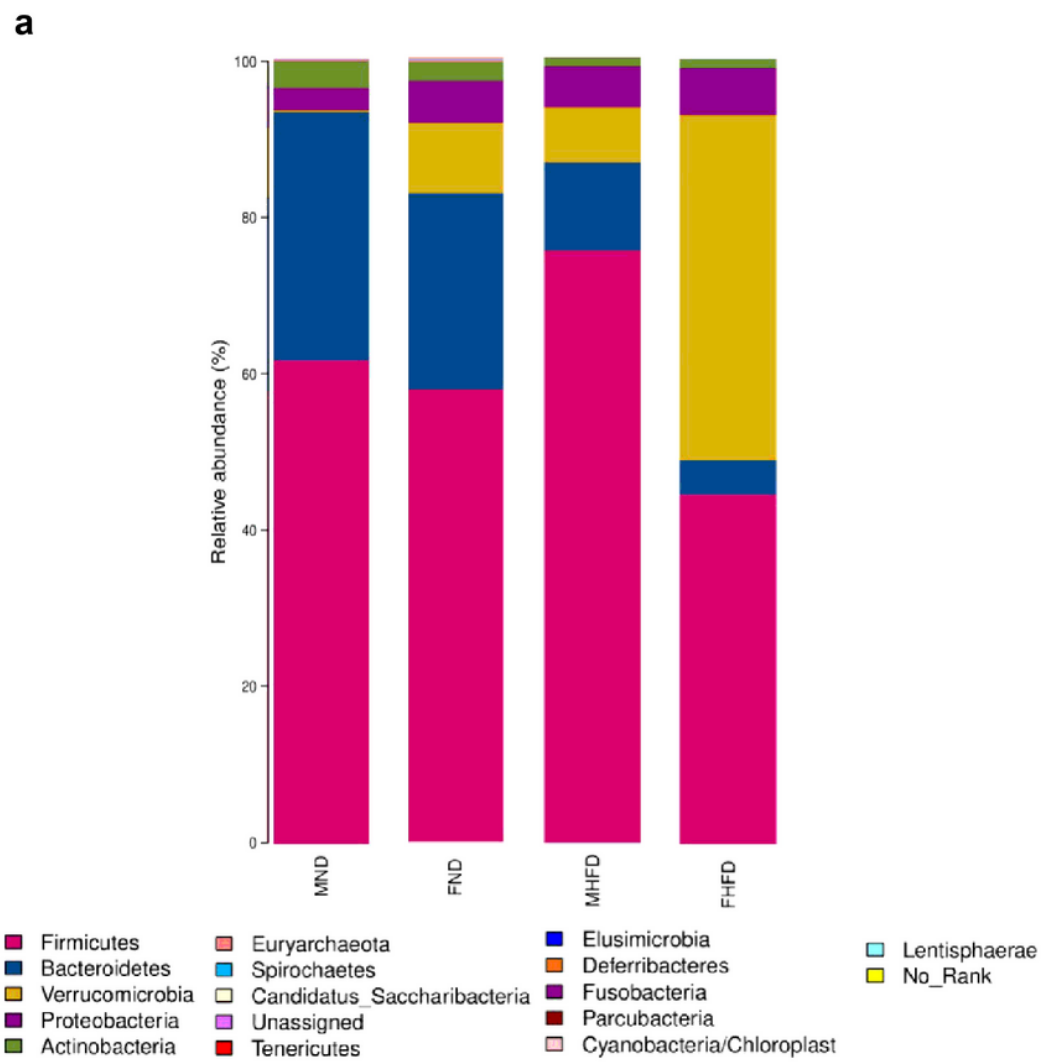

Figure 3

Effects of HFD on the microbial composition in rats of both genders. (a) Relative abundance of the microbial composition after HFD at the phylum level. (b, c and d) Changes in relative abundances of Firmicutes, Bacteroidetes and Verrucomicrobia. Data are expressed as mean ± SEM. a1p<0.05, a2p<0.01 vs MND group; b1p<0.05, b2p<0.01 vs FND group; c1p<0.05, c2p<0.01 vs MHFD group. Statistical significances were determined by one-way ANOVA and non-parametric test.

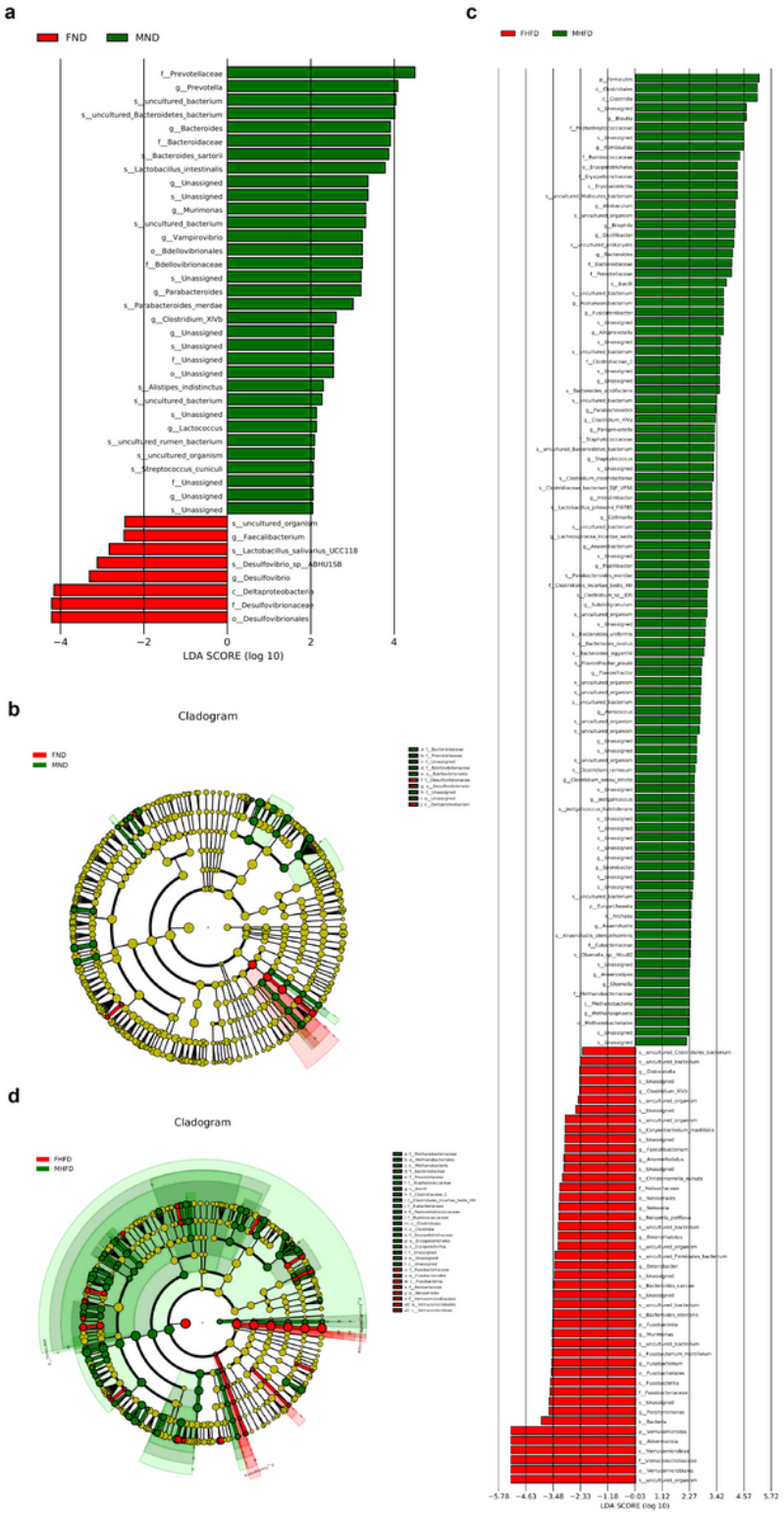

Figure 4

(a) LEfSe analysis between MND and FND group shows differentially abundant taxa as biomarkers determined using Kruskal-Wallis test with LDA score > 2.0. (b) Cladogram from LEfSe results between MND and FND group. (c) LEfSe analysis between MHFD and FHFD group with LDA score > 2.0. (d) Cladogram from LEfSe results between MHFD and FHFD group.

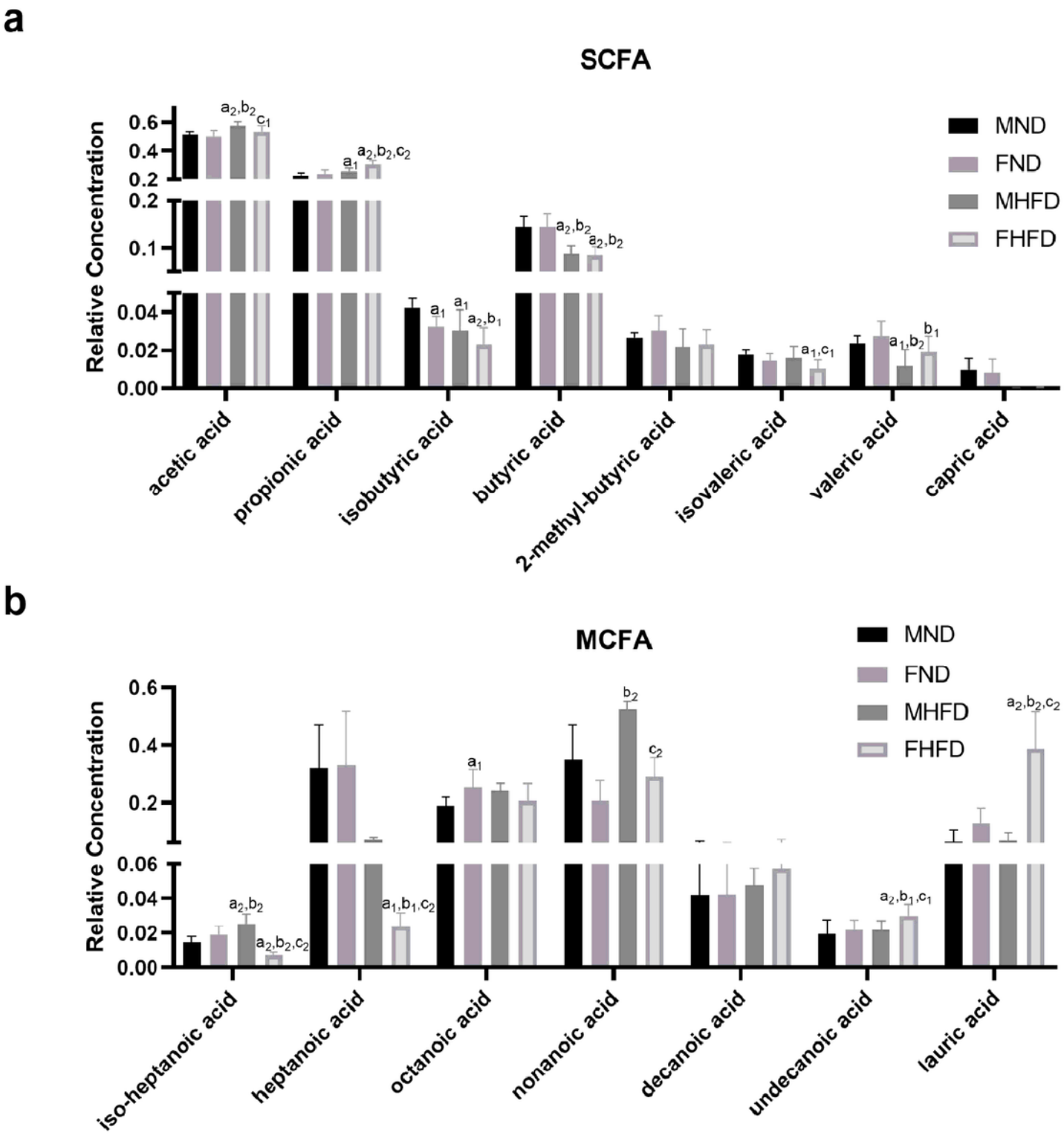

Figure 5

Sex differences in SCFAs and MCFAs of colon contents in ND of HFD-fed rats. (a) SCFAs of colonic contents. (b) MCFAs of colonic contents. Data are expressed as mean ± SEM. a1p<0.05, a2p<0.01 vs MND group; b1p<0.05, b2p<0.01 vs FND group; c1p<0.05, c2p<0.01 vs MHFD group. Statistical significances were determined by one-way ANOVA and non-parametric test.

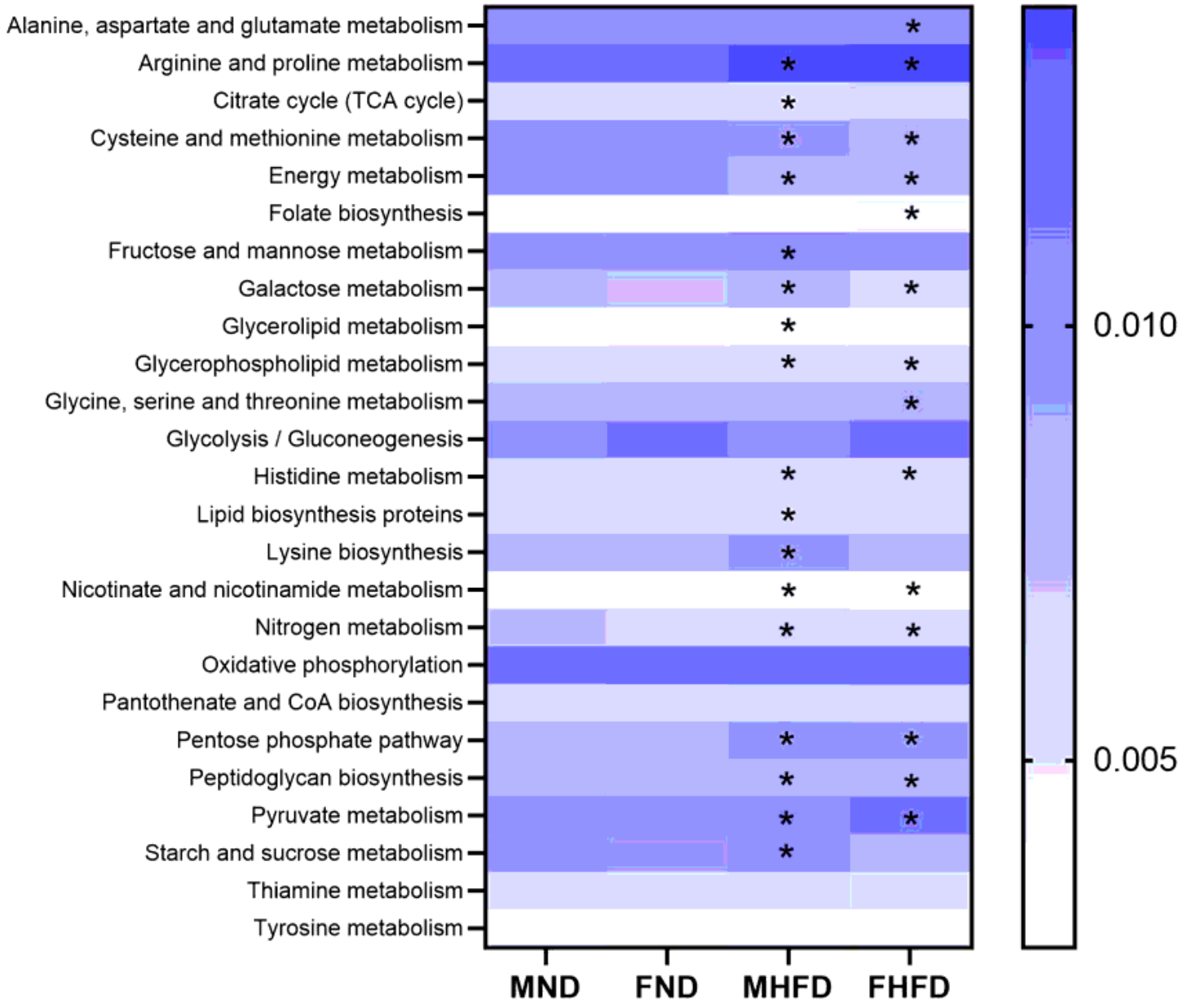

Figure 6

Heatmap of KEGG pathways predicted using PICRUSt. *p<0.05 compared with their sex-matched control groups.

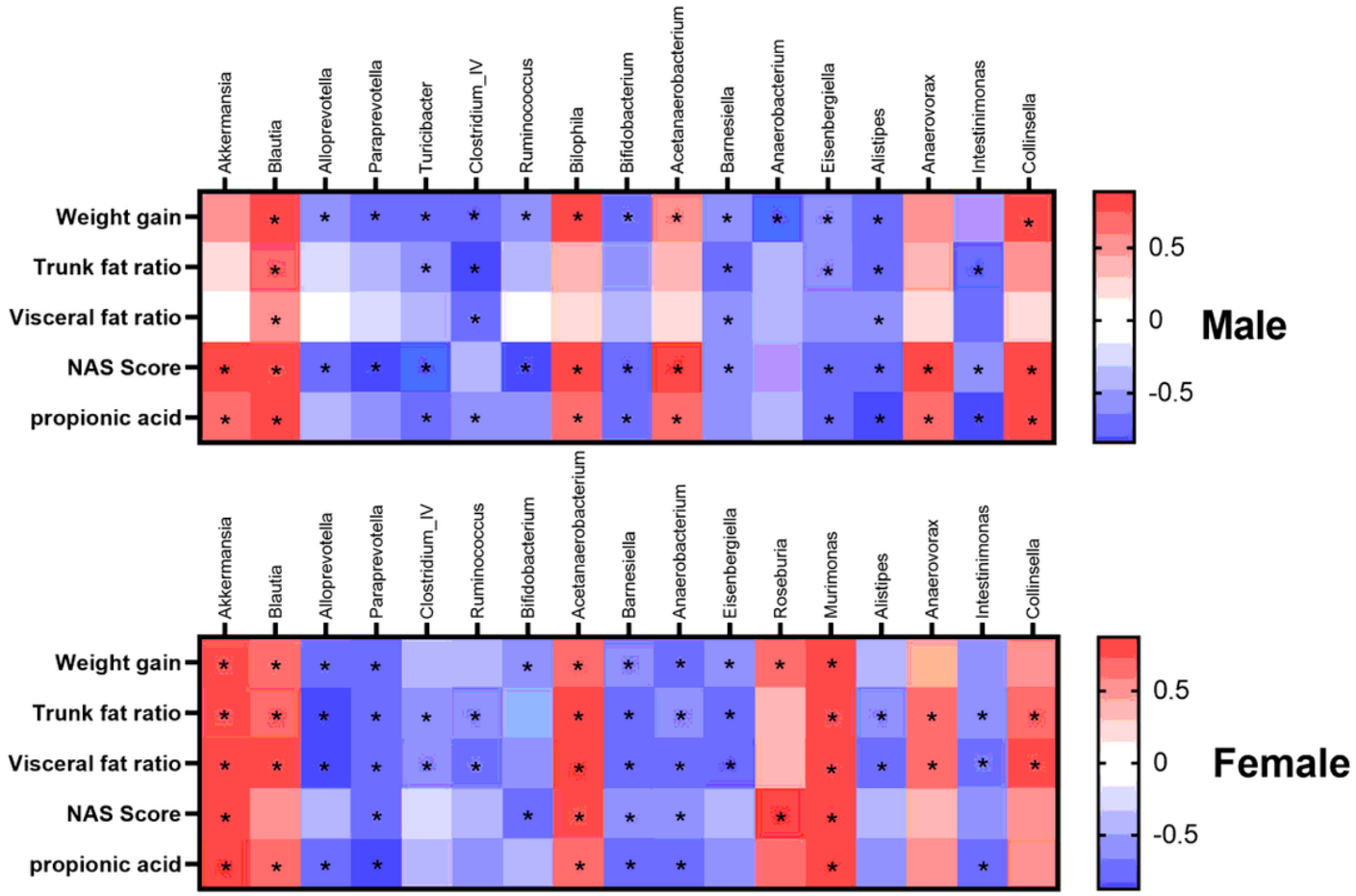

Figure 7

Correlation between key genera and phenotypes in rats of both genders. Red designates a positive correlation while blue designates a negative correlation. *p<0.05.

Supplementary Files

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- Additionalfile1.docx