Sex differences in gut microbiome in high-fat-diet fed rats.

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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. **Results** Sprague–Dawley rats were divided into the following groups (seven 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). 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 and Bilophila genus was detected in MHFD rats, as compared with FHFD, which showed increased relative abundance of Murimonas and Roseburia . Moreover, propionic and lauric acid levels were higher in FHFD than those in MHFD rats. **Conclusion** HFD induced sex-related alterations in gut microbiome and fatty acids. Furthermore, the genus Bilophila and Roseburia 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 obese². 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 hips³. Furthermore, prevalence of related metabolic diseases, such as type 2 diabetes and NAFLD, is higher in men than that in women⁴. 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 diseases⁵. In fact, there is growing evidence showing gut microbiota involvement in energy regulation, nutrient absorption, and fat storage^{6,7}. Germ-free mice have reduced adiposity when compared with colonized counterparts⁸, which protects them from high-fat-diet (HFD)-induced obesity^{9,10}. In addition, microbiota from an obese mouse may promote weight gain in a germ-free recipient mouse¹¹ that confirmed the link between host metabolism and gut microbiota.

Gender is an important factor altering gut microbiota. In this regard, several studies have provided evidence for gender differences in microbiota composition^{12,13}. 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 females¹⁴. In addition, a large Chinese cohort study demonstrated that BMI-associated differences in gut microbiota composition was influenced by gender¹⁵. HFD is commonly used to establish a model of obesity and metabolic syndrome in rodents¹⁶, and prolonged HFD in rats model mimics human obesity and obesity-related metabolic diseases. 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.

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 (Figure 1a). As shown in Figure 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 (Figure 1c). No significant differences in metabolic efficiency were observed between MHFD and MND, whereas HFD increased metabolic efficiency in female rats, which correlated with their body weight gain percentage induced by HFD (Figure 1d).

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 (Figure 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 (Figure 2a). The observed species and taxon diversity (Shannon index) of HFD groups were significantly reduced related to control levels (Figure 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 (Figure 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 (Figure 3a). A significant increase of *Firmicutes* was detected in MHFD compared with MND, but we found relative low abundance of *Firmicutes* after HFD feeding in female rats (Figure 3b). *Bacteroidetes* were found significantly less abundant in MHFD as compared with controls, and a similar trend was observed in females (Figure 3c). Notably, despite observing contrasting changes of *Firmicutes*, the ratio of *Firmicutes* to *Bacteroidetes* was increased in MHFD and FHFD. Furthermore, FHFD rats had a significantly increased level of *Verrucomicrobia*, as compared with that of the MHFD group (Figure 3d).

Bacterial taxa differences among different groups.

Linear discriminant analysis effect size (LEfSe) algorithm was applied to identify key phylotypes responsible for differences between MHFD and FHFD, compared with controls (Figure 3e). A cladogram of significantly different taxa in order and family level abundance is shown in Figure 3f, and detailed results at genus level are presented in Table 1. Among key genera with a relative abundance > 1% in at least one group, *Acetanaerobacterium*, *Blautia*, and *Akkermansia* were significantly more abundant in the MHFD and FHFD groups, but more evident in MHFD, as indicated by fold-change. In contrast, the relative abundance of *Bifidobacterium*, *Paraprevotella*, *Alistipes*, *Eisenbergiella*, and *Ruminococcus* was reduced after HFD feeding in both sexes. Notably, high levels of *Bilophila* were found only in MHFD, whereas *Murimonas* and *Roseburia* were only higher in FHFD, as compared with ND groups.

Sex-dependent metabolic changes in HFD rats.

Fatty acids were measured in colonic contents, including SCFAs and MCFAs (Figure 4a, b). HFD groups revealed 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.

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.

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 (Figure 5); 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 Figure 6. In particular, 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 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 positive relationships with *Murimonas* and *Roseburia* in female rats. Some genera were positively correlated 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^{17,18}, but some found the opposite¹⁹. Such a discrepancy could be due to differences in diet composition or diet period. In the present study, we found female HFD-fed rats gained more body weight than male HFD-fed rats. Metabolic 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 metabolic efficiency was higher in FHFD, but had no significant change in MHFD, compared with controls. The higher metabolic efficiency in FHFD may contribute to body weight gain percentage induced by HFD in females.

We also found that female HFD-fed rats had less severity of hepatic steatosis and inflammation than male HFD-fed rats, which agrees with previous studies²⁰. It is well known that estrogen protects from adipose tissue inflammation and oxidative stress, and development of liver steatosis²¹. The age of rats in our experiment was equivalent to human puberty, thus their metabolic and sex hormone levels were in a developing stage. Sex hormones may then explain the gender differences in fatty liver.

Traditionally, sex differences in obesity susceptibility have been explained by the different effects of sex hormones, which influence many aspects of metabolism and related diseases²². Nowadays, gut dysbiosis plays a crucial role in mediating the interaction between consumption of HFD and development of obesity in host. Whether alterations of gut microbiota explain the gender difference in metabolism, as well as 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

in mice and humans that obesity is associated with a reduced diversity of microbiota^{23,24}. Therefore, low microbiome diversity in HFHD may partly be attributed to the body weight gain percentage in females on HFHD. We observed HFHD increased the relative abundance of *Firmicutes* in males, whereas female rats had decreased proportions of *Firmicutes* in HFHD groups, which was not reported in previous studies. However, *Firmicutes* to *Bacteroidetes* ratio was increased in MHFD and HFHD rats, which has been frequently associated with obesity²⁵. Moreover, we were surprised to detect high levels of *Verrucomicrobia* after HFHD in females, as compared with those in male rats. *Verrucomicrobia* was reported to improve glucose metabolism in mice²⁶, and recent systematic reviews found adults with obesity and T2DM had fewer *Verrucomicrobia* than healthy individuals^{27,28}. In contrast, another study observed that the proportion of *Verrucomicrobia* increased in the HFHD group^{29,30}, which agrees with our results. Furthermore, Tang et al.³¹ found that *Verrucomicrobia* was positively correlated with cholic acid, which significantly increased in male rats in the NAFLD model. However, the role of *Verrucomicrobia* in lipid metabolism and liver steatosis in female rats remains to be elucidated.

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 MHFD group, and it positively correlated with weight gain and NAS score in male rats. *Bilophila* is a lipopolysaccharide-producing bacterium, previously reported to increase in response to HFHD^{32,33}. *Bilophila wadworthia* was found to synergize with HFHD to promote intestinal barrier dysfunction and bile acid dysmetabolism, leading to hepatic steatosis³⁴. In the HFHD group, we did not observe higher *Bilophila* abundance, which may suggest that changes in *Bilophila* load contributes to the gender differences in NAFLD severity. In contrast, a significant increase of *Roseburia* was only detected in HFHD, which was positively correlated with weight gain and NAS score in female rats, but not in males. As a butyrate-producing bacterium, *Roseburia* was reported to be negatively correlated with body weight and serum lipids in Chinese adults³⁵. A previous study indicated that restoring of *Roseburia spp.* correlated with increasing host metabolic perturbations induced by HFHD feeding in male mice, including body weight gain and hepatic triglycerides³⁶. In fact, the gender difference of *Roseburia* was also reported by Santos-Marcos³⁷, who observed a higher abundance of *Roseburia* in men than in women, after three years of low-fat diet. Taken together, we suggest that *Bilophila* and *Roseburia* were the main genus contributing to the gender difference of body weight gain and hepatic steatosis in HFHD feeding rats.

We also investigated sex-related SCFAs and MCFAs alterations after HFHD feeding. HFHD groups were shown to significantly increase propionic acid, particularly, HFHD 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 inflammation³⁸. Furthermore, propionic acid was reported to reduce lipogenic enzymes expression in liver, resulting in decreased hepatic triglycerides³⁹ and prevention of NAFLD by suppressing fatty acid synthesis and oxidative stress⁴⁰. 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 host⁴¹, 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 patients⁴². We have considered that the high concentration of propionic acid in HFHD may 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 recent studies showed that lauric acid decreased induction of obesity in HFD-fed male rats⁴³ and prevented hepatic lipid accumulation⁴⁴. 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 HFHD. It demonstrated that HFD feeding modified the functional metagenome in a sex-specific way.

The present study had some limitations, including the lack of measuring rats 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 considering other gut microbiota metabolites. Finally, fecal microbiota transplantation was not performed to verify the causal relationship between particular bacteria and body weight gain of different sexes.

Conclusion

Our results revealed that HFD feeding exhibited sex-dependent effects on gut microbiome and host metabolism in Sprague-Dawley rats. In addition, we found two main genera, *Bilophila* and *Roseburia*, which may partly contribute to the gender differences in body weight gain and liver steatosis. Therefore, more attention should be given to gut microbiota composition to explain the sex differences of diet-induced obesity and hepatic steatosis. However, the present findings showed associations between changes in the microbiome and phenotype, without any direct validation. 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.

Methods

Animals.

Six-week-old male and female specific-pathogen-free (SPF) Sprague–Dawley rats (n = 14 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 = 7 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 °C;

humidity, 55% $\pm$ 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; 3.5kcal/g) or HFD (37% fat, 17.5% protein, and 45.5% carbohydrate; 4.50 kcal/g) 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°C, until use. All rats used for tissue collection were euthanized by overdose intraperitoneal pentobarbital⁴⁵. 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 ballooning⁴⁶.

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 $\times$ 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. 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. 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.

Determination of fatty acids.

Fecal samples were stored at -80 °C until analysis. Fatty acids metabolites were extracted from fecal 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 °C, ion source gas 1 = 35, and ion source gas 2 = 5.

Statistical analysis.

Values were expressed as mean $\pm$ 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.

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.

Conflict of interest

The authors do not have any disclosures to report.

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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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Tables

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

	MND	MHFD	<i>p</i> -value; Fold Change	FND	FHFD	<i>p</i> -value; Fold Change
Alloprevotella	5.31±1.27	1.23±0.52	<i>p</i> >0.05;0.23	3.65±1.12	0.07±0.06	0.007;0.02
Bifidobacterium	2.01±1.46	0.02±0.01	0.014;0.01	1.31±1.17	0	0.020
Paraprevotella	4.28±0.70	0.55±0.25	0.017;0.13	2.46±0.86	0.05±0.05	0.005;0.02
Prevotella	3.94±0.78	1.64±1.00	0.033;0.42	1.45±0.31	0.58±0.56	<i>p</i> >0.05;0.4
Turicibacter	3.04±0.93	0.77±0.16	0.010;0.25	1.56±0.43	0.61±0.22	<i>p</i> >0.05;0.39
Acetanaerobacterium	0	1.54±0.26	<i>p</i> <0.001	0.05±0.05	0.57±0.07	0.045;11.4
Bilophila	0.09±0.02	2.98±0.81	0.003;33.11	0.17±0.06	0.13±0.06	<i>p</i> >0.05;0.76
Blautia	0.94±0.43	14.86±2.07	0.001;15.81	1.17±1.14	6.63±1.06	0.021;5.67
Alistipes	0.76±0.30	0.08±0.04	0.008;0.11	1.03±0.46	0.04±0.02	0.019;0.04
Anaerobacterium	0.81±0.24	0.32±0.18	<i>p</i> >0.05;0.40	1.09±0.26	0.02±0.01	0.001;0.01
Barnesiella	0.69±0.14	0.34±0.17	<i>p</i> >0.05;0.49	1.15±0.39	0.12±0.09	0.003;0.10
Eisenbergiella	1.01±0.33	0.02±0.00	0.001;0.02	1.12±0.51	0.09±0.03	0.041;0.08
Lactobacillus	4.78±0.93	1.20±0.23	0.028;0.25	6.26±3.08	0.68±0.36	0.009;0.11
Ruminococcus	1.58±0.41	0.19±0.08	0.006;0.12	2.21±0.65	0.34±0.14	0.028;0.15
Akkermansia	0.27±0.21	7.01±2.86	0.034;25.96	8.97±5.65	43.89±3.83	0.004;4,89
Murimonas	0.50±0.11	0.33±0.05	<i>p</i> >0.05;0.66	0.06±0.05	1.05±0.20	<i>p</i> <0.001;17.5
Roseburia	0.25±0.08	0.44±0.17	<i>p</i> >0.05;1.76	0.17±0.04	1.15±0.39	0.007;6.76

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.

Supplemental Information Note

Additional file 1.docx contains ARRIVE guidelines checklist.

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) Metabolic efficiency (body weight gain divided by the total energy intake). (e) Trunk fat ratio. (f) Visceral fat ratio. (g) Representative pictures of H&E staining in liver tissue (200×). (h) NAFLD activity Score. Data are expressed as mean $\pm$ 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 diagrams shared OTUs among groups. (b) Observed species. (c) Shannon index. (d) PCoA analysis based on jaccard distance. Data are expressed as mean $\pm$ 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.

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. (e) LefSe analysis shows differentially abundant taxa as biomarkers determined using Kruskal-Wallis test with LDA score > 2.0. (f) Cladogram from LefSe results between different groups. Data are expressed as mean $\pm$ 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

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 $\pm$ 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 5

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

Figure 6

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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