A potential probiotic- *Lachnospiraceae NK4A136 group*: Evidence from the restoration of the dietary pattern from a high-fat diet

Menq-Rong Wu
National Taiwan University https://orcid.org/0000-0002-6139-8062
Te-Sen Chou
Taipei Tzu Chi Hospital
Ching-Ying Huang
National Chung Hsing University
Jong-Kai Hsiao (jongkai@gmail.com)
Department of Medical Imaging, Taipei TzuChi General Hospital, Buddhist Tzu-Chi Medical Foundation

Research article

Keywords: High-fat diet, Dietary pattern shift, Dysbiosis, Akkermansiaceae, Lachnospiraceae NK4A136 group, Gut microbiota

Posted Date: August 4th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-48913/v1

Abstract

Background: High-fat diet (HFD) that contributes to obesity is one of the pivotal risk factors for metabolic syndrome and cancers. The dietary pattern can shape the intestinal bacterial community and influence the physiological parameters. This study aimed to investigate whether the short-term dietary pattern shift from HFD to a balanced chow diet (CD) could correct HFD-induced colonic dysbiosis and reverse adverse health effects and identify the specific bacteria that changed by dietary patterns.

Results: C57BL/6 mice fed with an HFD for 10 months, followed by a CD for 3 months, served as the dietary shift model. Stool samples were collected for bacterial analysis. Physiological parameters, such as serum adipokines, blood lipid levels, and hepatic function, were monitored in control and dietary shift groups. HFD-induced weight gain was mitigated by the dietary shift. A highly similar structure at the phylum, genus, and species levels was observed in the beta diversity of colonic bacteria in mice that underwent the dietary shift as compared to those in the control group. Notably, the abundance of *Peptococcaceae* and *Akkermansiaceae* in HFD-fed mice reduced after the dietary shift; whereas the diminished amount of probiotic *Lachnospiraceae NK4A136 group* in HFD-fed mice was restored to the level comparable to those in controls after the dietary shift.

Conclusions: Our finding suggests that a dietary switch from a long-term HFD to a short-term balanced diet has the potential to correct colonic dysbiosis and restore physiological homeostasis. The *Lachnospiraceae NK4A136 group* has the potential to be a probiotic.

Background

In developed countries, the overconsumption of dietary fat is the most common cause of obesity, metabolic syndrome, and several chronic diseases. Studies have revealed that the high-fat diet (HFD)-induced intestinal dysbiosis is associated with a decrease in mucus thickness and low-grade inflammation. The consumption of HFD lowers the expression of tight junctions in the intestinal epithelium, which has been associated with subsequent obesity, insulin resistance, and hyperglycemia in both human and animal models [1, 2]. HFD could also adversely affect the brain by impairing cognitive functions or promoting depression-like symptoms [3–5]. Several animal studies have demonstrated that dietary shifts from HFD to a balanced diet could correct adverse effects resulted from HFD with permanent or transgenerational benefits [6, 7].

Recently, several studies have reported the interaction between the alteration of dietary fats and intestinal microbiota [8–10]. The human intestine harbors approximately 10^{14} bacteria with over 1000 species [11, 12]. Gut commensal bacteria play a crucial role in the maintenance of immune responses, renewal of the intestinal epithelium, and regulation of metabolic homeostasis [13–16]. Alteration of the composition of gut microbiota has been linked to the development of obesity [17, 18]. Many studies have reported a reduction in bacterial diversity after an HFD [19–22]. In rodent models of obesity, mice fed with HFD for 3–6 months were associated with the change in gut microbiota [23]. Increasing evidence from animal

and human studies have revealed a higher *Firmicutes* to *Bacteroidetes* (F/B) ratio in the gut of obese individuals [17, 19, 20]. Furthermore, a lowered abundance of *Proteobacteria* has been found after HFD exposure [20–22, 24]. However, HFD-induced alteration of gut microbiota could be modified by body weight control [19], beta-glucan [25] or pectin -containing diet dietary [26], metformin [20], or probiotics interventions such as *Bacteroides uniformis CEST 7711* [23] or *B. pseudocatenulatum* [21]. Whether the short-term dietary pattern shift from HFD to a balanced diet could correct HFD-induced colonic dysbiosis and reverse adverse physiological alterations still requires further investigation. The lack of fiber intakes is also associated with the HFD-induced gut dysbiosis [27, 28]. The short-chain fatty acid (SCFA)-producing bacteria such as *Lachnospira*, *Akkermannsia*, *Bifidobacterium*, *Lactobacillus*, *Ruminococcus*, *Roseburia*, *Clostridium*, *Faecalibacterium,* and *Dorea* was a significant decrease in human receiving low fiber diet [29, 30].. In addition, intestinal *Bacteroides acidifaciens* were increases in mice fed a high fiber diet [31] and played a role in obesity prevention and improves insulin sensitivity in mice [32]. Notably, HFD has also been linked to enhanced tumorigenicity of colonocytes [33, 34]. A possible mechanism is the disruption of the colonic mucosal barrier, which increases the adherence of pathogenic bacteria mediated by HFD-induced dysbiosis [35]. The increase of fiber mucus-degrading bacteria, *Akkermansia muciniphila*, was found in mice fed with a fiber-deficient [36] or red meat-mimic heme diet [37], and resulted in the pathogenic bacterial adhesion and invasion to the colonic mucosa [36] or caused epithelial hyperproliferation [37]. However, whether the mucus-degrading bacteria also contribute to HFD and dietary pattern shift is still unknown.

Lachnospiraceae NK4A136 group, which is belonged to the family of *Lachnospiraceae*, was noticed recently. *Lachnospiraceae* is characterized by the anaerobic and spore-forming features with the ability to ferment the plant polysaccharides into SCFAs and ethanol [38]. The role of *Lachnospiraceae NK4A136 group* in diet-associated metabolism is still controversial. In HFD mice, the numbers of *Lachnospiraceae NK4A136 group* were reduced and negatively correlated to triglyceride levels [39]. The elevation of *Lachnospiraceae NK4A136 group* was found in mice fed with *Gracilaria Lemaneiformis*-derived sulfated polysaccharide and positively correlated to the secretion of bile acids [40]. Moreover, obese mice with fucosylated chondroitin sulphate feeding showed the anti-inflammation effects as well as the elevation of *Lachnospiraceae NK4A136 group* [41]. However, Liu *et al.* have reported that the abundance of *Lachnospiraceae NK4A136 group* did not affect in high-fat diet-fed mice [40]; Zhang *et al.* demonstrated that the increase of *Lachnospiraceae NK4A136 group* in type 2 diabetes mellitus rats and the bacterial level returns back to control after fed with anthraquinone-glycoside [42]. Whether the *Lachnospiraceae NK4A136 group* level could be modified by dietary fat content remains elusive.

In the current study, we aimed to (1) evaluated the effects of both short-term (3 months) and long-term (10 months) HFDs on biological changes and colonic bacterial alteration, (2) investigated the therapeutic potential of a short-term (3 months) dietary pattern shift from an HFD to a balanced CD in terms of modulation of the intestinal bacterial community, and (3) identified specific bacteria responsible for distinct dietary effects.

Results

HFD contributed to the elevation of body weight and alteration in the bacterial community in mice

Mice were fed with either a control balanced CD or an HFD for the indicated period (Fig. 1a). The percentage of calories from fat was 12% in the CD and 62% in the HFD. Compared with controls, the average body weight of mice fed with the HFD for 3 and 10 months increased significantly by 1.30- and 1.29-fold, respectively (Fig. 3a and 3d). Colonic stool samples were collected at the indicated time points, and the diversity of the bacterial community was investigated. The taxonomic profiles of bacterial beta-diversity were analyzed using PCA. Using weighted UniFrac to analyze the beta diversity, the distribution of points related to both short-term HFD (SHFD) and long-term HFD (LHFD) samples were compared with those of the control group. HFD-related clusters were close to PC1, which accounted for 73.2% of the total variation, indicating that the HFD had a considerable effect on the colonic bacterial community in mice (Fig. 4a). A similar cluster distribution was also found in the lower-level classification of bacterial taxonomy in terms of genus and species, suggesting an alteration of bacterial diversity in mice fed with either an SHFD or an LHFD (Fig. 4b and 4c).

Effects of HFD and dietary shift on gut microbiota alteration

- **Obesity-associated gut bacterial changes were found in mice with both SHFD and LHFD consumption**

The relative abundance of several bacteria taxa was further analyzed to investigate the contribution of diet in modulating the bacterial community of the colon. An increased F/B ratio is known to be associated with obesity. Stool samples derived from both SHFD and LHFD mice revealed that the composition of bacteria at the phylum level was altered in the DS group; major changes were observed in Bacteroidetes, Firmicutes, Verrucomicrobia, and Patescibacteria (Fig. 5a). A significant decrease in the abundance of Bacteroidetes was noted (Fig. 5b), whereas no statistically significant difference was observed in the number of Firmicutes and the F/B ratio in mice fed with the different diet (SHFD vs. C-SHFD, $p = 0.13$; LHFD vs. C-LHFD, $p = 0.10$; Fig. 5c–d). In addition, the phyla Verrucomicrobia and Patescibacteria were relatively abundant in the stool sample from mice fed with LHFD.

At the lower taxonomic level, further analysis was conducted to elucidate which bacterial families were affected after HFD consumption. Compared with controls, *Peptococcaceae* (Fig. 8a) and *Akkermansiaceae* (Fig. 8b) were significantly increased in the stool samples of mice fed with either an SHFD or an LHFD. However, the elevation of *Tannerellaceae* only appeared in mice fed with an SHFD, not in mice fed with an LHFD (Fig. 8c). Our finding suggests a correlation between HFD-induced colonic dysbiosis and obesity in mice.

Moreover, we further differentiated the composition of the characteristic microbes in species by linear discriminant analysis effect size (LEfSe). *Bacteriodes acidifaciens* and *peptococcaceae* were abundant in SHFD and LHFD groups (Fig. 6a-d). On the other hand, *Lachnospiraceae NK4A136 group* was abundant in C-SHFD and C-LHFD groups (Fig. 6a-d). After fed with CD, *Bacteriodes acidifaciens* was still abundant in SHIFT group (Fig. 7 and 8d). The proportion of *Lachnospiraceae NK4A136 group* was reduced while fed with HFD (p=0.056 at 10th month) and gradually increased to the level as NDC group (Fig. 8e).

- **The short-term dietary shift corrected HFD-induced weight gains and colonic dysbiosis to regular balance chow diet (CD)**

The composition of gut bacteria could be modulated by dietary shifts, such as the alteration of macronutrient composition, total calories, or specific supplements. To evaluate the effect of a short-term dietary shift from an HFD to a regular CD on the recovery of colonic bacteria composition and obesity-related parameters, mice fed with an LHFD were shifted to a CD for 3 months. During the 3-month period, the weight gain of the DS mice was comparable to that of the NDC mice (Fig. 3c). The circulating levels of adipokine, leptin, and adiponectin were not significantly different between groups, suggesting the restoration of adipocyte homeostasis after the dietary shift (Fig. 3d and 3e).

PCA revealed that stool samples collected from the mice that shifted to a CD for 3 months after HFD feeding for 10 months (SHIFT mice) displayed a smaller difference along PC2, which accounted for 10.9% of total variations at the phylum level (Fig. 4a). A similar pattern of cluster distribution was also found in the genus and species levels. These results suggest that the short-tern dietary shift corrected the HFD-induced alteration in bacterial diversity. The effect of dietary shift on bacterial taxa was further investigated. Compared with controls, the abundance of Firmicutes (Fig. 5b) and Bacteroidetes (Fig. 5c) as well as the F/B ratio (Fig. 5d) were not significantly different in mice after the short-term dietary shift, indicating that obesity-associated dysbiosis was corrected by the shift to a normal diet. In addition, an HFD led to elevated Verrucomicrobia (Fig. 5e) and Patescibacteria (Fig. 5f) abundance, which decreased after the dietary shift; no significant difference was noted between groups. Similar results in the family level revealed no significant difference in *Peptococcaceae* (Fig. 8a), *Akkermansiaceae* (Fig. 8c), and *Tannerellaceae* (Fig. 8b) between the DS and NDC groups. Moreover, the colon morphology of the SHIFT group was not different to that of the parallel controls of the LHFD mice (C-SHIFT group) (Fig. 8d and 8e)

No difference in blood lipid profiles and liver function parameters between groups after short-term dietary shift

HFD-induced abnormal blood lipid profiles and liver dysfunction are known risk factors for obesity-associated diseases. No significant difference in serum total cholesterol (TCHO), high-density lipoprotein (HDL), and triglyceride (TG) levels were noted between DS and NDC mice (Fig. 9a). In terms of liver function, serum glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, and total bilirubin were not notable different between groups after the short-term dietary shift (Fig. 9b). The hepatic tissues

of NDC and DS mice were processed for H&E staining. Neither group exhibited signs of pathological defects (Fig. 9c).

Discussion

Our results provided evidence that (1) HFD-induced colonic dysbiosis is associated with weight gain, which was significantly reversed by the short-term dietary switch from an HFD to a normal balanced CD; (2) both an SHFD or an LHFD could lead to colonic dysbiosis with an increase in the abundances of *Peptococcaceae* and *Akkermansiaceae* families; (3) the increased in the abundance of *Bacteriodes acidifaciens* and decreased of *Lachnospiraceae NK4A136 group* in the DS. (Fig. 3a-c); (4) No changes in blood lipid profiles and liver function were found in DS mice in comparison with the control group. These results suggest that the correction of HFD-induced dysbiosis through dietary modification may be a pivotal factor for the maintenance of physiological homeostasis, and the potential health problem caused by obesity could be partially resolved by dietary changes.

The formula of HFDs was diverse and affected microbiota differentially [43]. In general, obesity was linked to a 50% reduction in the abundance of *Bacteroidetes* in mice and humans [17, 19, 20]. Furthermore, this change was reversed after weight loss [19]. A HFD can increase *Firmicutes* and decrease *Bacteroidetes* in the human gut [44]. Tang *et al* have been reported that the abundances of *Erysipelotrichaceae*, *Family_XIII*, *Ruminococcaceae*, *ratAN060301C*, *Clostridiales Coprococcus*, *Intestinimonas*, *Parabacteroides*, *Pseudobutyrivibrio*, and *Roseburia* are increased after HFD feeding. Some microbes such as *Defluviitaleaceae*, *Defluviitaleaceae*, *Lachnospiraceae*, *Peptococcaceae*, *vadinBB60, Christensenellaceae*, *Coriobacteriaceae*, *Peptostreptococcaceae*, *Prevotellaceae*, *RF9*, *Ruminococcaceae*, and *S24-7* (*Muribaculaceae*) are decreased after a HFD feeding [45]. *Ruminococcus, Akkermannsia*, *Bacteroidetes, Faecalibacterium*, *Bifidobaterium*, *Lactobacillus* and *Blautia* are positively corelated to type 2 diabetes. Furthermore, *Rumonococcus* and *Fusobacterium* are negatively correlated to type 2 diabetes [46]. These microbes involves in the gut permeability, metabolism, and inflammation in directly or in directly way [46]. In our study, we also found that the decreased proportions of *Bacteroidetes*, and *Muribaculaceae* and the increased proportions of *Akkermansia, Parabacteroides*, *Intestinimonas*, and *Roseburia* (Fig. 6a-d and Additional file 1) indicating our HFD feeding was workable. However, the abundance of *Peptococcaceae* was increased in SHFD and LHFD which might because of the differences of HFD formula (Fig. 6a-d, 7a) [43]. In the current study, we found that the diet shifting could restore an imbalanced microbiota. However, some genera were still affected even after the dietary shift, such as *Acetatifactor*, *Ruminiclostridium*, *GCA−900066575*, *Ruminiclostridium 5*, *Ruminococcaceae UCG−004*, *Lachnospiraceae UCG−006*, *Intestinimonas*, and *Lachnospiraceae AC2044* group labeled as C2 (Additional file 1). Some genera might also be affected by the dietary shift, such as *Roseburia*, *Anaerotruncus*, *Oscillibacter*, *Marvinbryantia*, and *Lachnospiraceae UCG−001* labeled as C4 (Additional file 1). The functions of these genera remain unclear at present. They might play roles in the homeostasis of nutrition, metabolism, and even the immune system. Further study on their functions is warranted.

The function of *Lachnospiraceae NK4A136 group* is not clear. In HFD mice, *Lachnospiraceae NK4A136 group* is reduced and negatively correlates to triglyceride [39]. Instead, Liu *et al* have been reported that the abundance of *Lachnospiraceae NK4A136 group* has no effect at high-fat diet-fed mice; however, it elevates after fed with a sulfated polysaccharide from *Gracilaria Lemaneiformis* [40]. The diverse data might be due to the ingredients of HFD and the duration for HFD feeding. *Lachnospiraceae NK4A136 group* positively correlates to bile acids indicating it involving in cholesterol homeostasis [40]. Zhang *et al* have found that the abundance of *Lachnospiraceae NK4A136 group* increases in type 2 diabetes mellitus rat and returns to the level as the control after fed with anthraquinone-glycoside [42]. Moreover, obese mice with fucosylated chondroitin sulphate feeding showed the anti-inflammation effects as well as the elevation of *Lachnospiraceae NK4A136 group* [41]. In our study, the abundance of *Lachnospiraceae NK4A136 group* decreased during HFD feeding and returned to the level as NDC mice after fed with CD (Fig. 6 and 7e) suggesting that it might be a potential probiotic. *Lachnospiraceae NK4A136 group* needs further investigation in the following study.

High fiber dietary is a factor to influence gut microbiota and produce SCFAs to regulating metabolism by gut microbiota [47]. High fiber diet significantly elevated microbes, involving SCFA production, such as *Lachnospira*, *Akkermannsia*, *Bifidobacterium*, *Lactobacillus*, *Ruminococcus*, *Roseburia*, *Clostridium*, *Faecalibacterium,* and *Dorea* [30]. In our study, the proportions of *Bacteriodes acidifaciens* and *Akkermansiaceae* family were increased significantly in mice fed with either an SHFD or an LHFD (Fig.6a-d, 8c, and 8d). Moreover, a 3-month CD shift could not restore the level of *Bacteriodes acidifaciens* (Fig. 7 and 8d). *Bacteriodes acidifaciens* has been reported that it prevents obesity and improves insulin sensitivity [32] and its abundance elevates in the high fiber dietary [31, 48]. In our study, CD dietary contained 5.3 % crude fiber, 15.4 % neutral detergent fiber, and 6.3 % acid detergent fiber. The fiber in HFD was 6.5 % and derived from powdered cellulose. The composition of fiber was different between CD and HFD. The abundance of *Bacteriodes acidifaciens* was increased in the DS group might be affected by the fibers or HFD (Fig. 6 and 8d). After the dietary shift, abundance of *Bacteriodes acidifaciens* was still significantly higher in the DS group (Fig. 7 and 8d). It indicates that *Bacteriodes acidifaciens* was not easy to be affected by CD. he mucus-degrading *A. muciniphila,* which belongs to the *Akkermansiaceae* family, utilizes colonic mucus as a carbohydrate source and is associated with increased pathogen susceptibility through enhanced bacterial colonization in the epithelium when mice were fed with a fiber-free diet [36]. *A. muciniphila* also increased in mice receiving a red meat–mimic heme diet, leading to colonic mucolysis [37]. An HFD as well as red meat-free and fiber-free diets are risk factors for colorectal cancer; however, the mechanism is under-investigated. Although reports have indicated that increased *A. muciniphila* may have beneficial effects on obese mice,[49] our study provides new insights into the role of *Akkermansiaceae* bacterial in mice receiving an HFD. At the endpoint, the lipids level and adipokines were similar between the NDC and DS groups (Fig. 3d, 3d, 9a-c). *Bacteriodes acidifaciens* and *Akkermansiaceae* family might help the homeostasis of lipids level and adipokines [32].

An HFD has several effects on animal anatomy and physiology and results in pathological changes. Although HFD-fed mice exhibited increased blood lipids, their liver function was not affected [50]. In the current study, histology of the liver revealed similar morphologies in the DS and NDC mice (Fig. 9g). The

DS and NDC mice also exhibited similar blood lipid levels. Moreover, although TCHO, HDL, and TG levels decreased in the DS mice, this decreased level was not significant (Fig. 9a-c). We further examined adipokines, leptin, and adiponectin (Fig. 3d and 3e). Studies have reported elevated leptin and unchanged adiponectin in the serum of HFD-fed mice [51, 52]. The results of the present study indicated that gut length and blood lipid levels were restored in the DS mice.

Despite these findings, our study has some limitations. Sample sizes for determining the microbiota pattern after LHFD and dietary shifts were small (N = 3 for NDC and N = 5 for DS). The mice were maintained in a relatively simple environment, which enabled their physiology and microbiota to be restored more through the dietary shift to a regular balanced diet. In this study, we only investigated the dominant bacteria. Some minor species that play critical roles may have been overlooked. However, we attempted to provide an overall observation of the condition of the DS group. Hopefully, this information can inspire researchers to investigate therapies to counteract the effects of HFD.

Conclusion

Our results revealed that a short-term dietary shift could modulate colonic dysbiosis caused by an HFD and maintain physiological homeostasis. Therefore, nutritional intervention is an effective tool to prevent the adverse health problem associated with an HFD

Methods

Animals and experimental design

Male C57BL/6 mice aged 6–8 weeks were purchased from the National Laboratory Animal Center and were raised at the animal center in Taipei Tzu Chi Hospital. All experimental procedures were approved by the Institutional Animal Use and Care Committee of Taipei Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation (105-IACUC-007). Mice were maintained according to the recommendations of the Guide for the Care and Use of Laboratory Animals (National Institutes of Health). At age 10 weeks, the mice were randomly assigned into 2 groups. Group 1, the normal balanced diet control (NDC; N = 3) group was fed with a CD for 13 months. The formula for the CD (Prolab RMH2500 5PI4, LabDiet, St. Louis, MO, USA) was 12.1% fat, 28.8% protein, and 59.1% carbohydrates to produce calories (Fig. 1a and 1b). Group 2, the dietary shifting (DS) group (N = 5), was fed with an HFD for 10 months, followed by a balanced CD for 3 months. The diet was acquired by itself. The formula for the HFD (58Y1, TestDiet) was 61.6% fat, 18.1% protein, and 20.3% carbohydrates to produce calories (Fig. 1a and 1b). CD dietary contained 5.3 % crude fiber, 15.4 % neutral detergent fiber, and 6.3 % acid detergent fiber. The fiber in HFD dietary was 6.5 % and derived from powdered cellulose. Each group was further divided into 3 subgroups at 3–3.5 (SHFD), 10 (LHFD), and 13 (SHIFT) months. The parallel controls were named C-SHFD, C-LHFD, and C-SHIFT at 3–3.5, 10, and 13 months, respectively. The bodyweight of each mouse was recorded once weekly, and stool samples were collected at six time points (3-3.5, 7, 10, 11, 12, and 13 months) during the distinct diet

exposure (Fig. 2). Mice were euthanized at 13 months by using isoflurane (Rhodia Organique Fine, Bristol, UK). All animal experiments were performed in the animal room.

Blood Sampling and Stool collection

Blood samples collected from the hearts at the endpoint of the exam (at 13 months) were centrifuged at 3000 rpm for 15 min at 4 °C for serum collection. Stool samples were collected in the morning; the stool in each cage had been removed the previous evening. The stool samples were stored at 4 °C before sequencing.

Cytokine analysis and blood chemistry analysis

Serum samples were used for cytokine analysis by using a Proteome Profiler Array Mouse XL Cytokine Array Kit (ARY028; R&D Systems, Minneapolis, MN, USA). In general, 200 µl serum samples were added into the antibody-coating membrane provided by the kit. The signals were detected by the BioSpectrum 810 Imaging System (UVP, Upland, CA, USA) and quantified by Image J software [53]. Remaining serum was analyzed with Fuji Dri-Chem 4000i in Taiwan Mouse Clinic for blood chemistry analysis.

Sequencing of 16S rRNA genes and data analysis

Bacterial DNA was extracted from fecal samples by using a QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany). After DNA extraction, we delivered the samples to Genomics (Taipei, Taiwan) for 16S rRNA gene sequencing on an ABI 3730XL DNA Analyzer (Applied Biosystems, Foster City, CA, USA). Data were analyzed using ClustVis software [54]. Beta diversity was analyzed using weighted UniFrac analysis principal component analysis (PCA) with all operational taxonomic units (OTUs). Diversity at the genus and species level were analyzed using principal component analysis (PCA) with OTUs excluding OTUs below 0.05 % after normalization. Heatmap was generated using the data at the genus level excluding OTUs below 0.05 % after normalization. The parameters used for PCA and heatmap were row centering, unit variance, SVD with imputation. Moreover, the heatmap was represented with collapsed columns-median. The significant biomarkers were analyze by linear discriminant analysis effect size (LEfSe) [55]. The parameters for LEfSe: alpha value for the factorial Kruskal-Wallis test among classes was 0.05 and the LDA scores were 4 at C-SHFD, SHFD, C-LHFD, and LHFD groups and 2 at C-SHIFT and SHIFT groups.

Histological analysis

Liver and colon tissues were collected and fixed in 10% formalin and embedded in paraffin wax. Five-µm-thick sample sections were deparaffinized and stained with hematoxylin and eosin (H&E). Histological slides were observed using an ECLIPSE TE2000-U microscope (Nikon, New York, NY, USA).

Statistical analysis

All data are expressed as the mean ± standard error and analyzed with GraphPad Prism 5 software. The NDC and DS groups were compared using Student's *t* test. Significance was established at $p < 0.05$.

Abbreviations

CD: chow diet; DS: dietary shifting; F/B: *Firmicutes* to *Bacteroidetes*; H&E: hematoxylin and eosin; HDL: high-density lipoprotein; HFD: high-fat diet; LEfSe: linear discriminant analysis effect size; LHFD: long-term HFD; NDC: normal balanced diet control; OTUs: operational taxonomic units; PCA: principal component analysis; SCFAs: short chain fatty acids; SHFD: short-term HFD; TCHO: total cholesterol; TG: triglyceride

Declarations

Acknowledgment

We are grateful to the Eighth Core Lab, Department of Medical Research, National Taiwan University Hospital, and the Core Lab, Taipei TzuChi General Hospital, Buddhist Tzu Chi Medical Foundation, for providing facility support.

This manuscript was edited by Wallace Academic Editing

Authors' contributions

MW performed all experiments and analysis and original draft preparation. TC involved all animal experiments. CH conceptualized the study and edited the draft. JH designed, conceptualized, and supervised the study and edited the draft. All authors have read and approved the manuscript

Funding

This research was funded by the Buddhist Tzu Chi Medical Foundation (TCRD-TPE-106-34) and the Ministry of Science and Technology of Taiwan (MOST 107-2314-B-303 -006 -MY3).

Availability of data and materials

All data related to microbiota analysis was included in additional files. Metagenomic sequencing data were deposited in the Mendeley Dataset (DOI: 10.17632/sbd96vtx45.1, reveal date: 2020/7/30)

Ethics approval and consent to participate

Treatment of Laboratory Animals and were reviewed and approved by the Institutional Animal Use and Care Committee of Taipei Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation (105-IACUC-007).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have not competing interests.

References

1. ZmoraN, SuezJ, ElinavE. You are what you eat: diet, health and the gut microbiota. Nat Rev Gastroenterol Hepatol. 2019;16:35–56. doi:10.1038/s41575-018-0061-2.
2. AraújoJR, TomasJ, BrennerC, SansonettiPJ. Impact of high-fat diet on the intestinal microbiota and small intestinal physiology before and after the onset of obesity. Biochimie. 2017;141:97–106. doi:10.1016/J.BIOCHI.2017.05.019.
3. ChianeseR, CoccurelloR, ViggianoA, ScafuroM, FioreM, CoppolaG, et al. Impact of Dietary Fats on Brain Functions. Curr Neuropharmacol. 2017;16:1059–85. doi:10.2174/1570159x15666171017102547.
4. DuanY, ZengL, ZhengC, SongB, LiF, KongX, et al. Inflammatory links between high fat diets and diseases. Front Immunol. 2018;9 NOV:2649. doi:10.3389/fimmu.2018.02649.
5. VagenaE, RyuJK, Baeza-RajaB, WalshNM, SymeC, DayJP, et al. A high-fat diet promotes depression-like behavior in mice by suppressing hypothalamic PKA signaling. Transl Psychiatry. 2019;9:141. doi:10.1038/s41398-019-0470-1.
6. DiMecoA, PraticòD. Early-life exposure to high-fat diet influences brain health in aging mice. Aging Cell. 2019;18:e13040–e13040.
7. SiddeekB, MauduitC, ChehadeH, BlinG, LiandM, ChindamoM, et al. Long-term impact of maternal high-fat diet on offspring cardiac health: role of micro-RNA biogenesis. Cell Death Discov. 2019;5:71.
8. WanY, WangF, YuanJ, LiJ, JiangD, ZhangJ, et al. Effects of dietary fat on gut microbiota and faecal metabolites, and their relationship with cardiometabolic risk factors: a 6-month randomised controlled-feeding trial. Gut. 2019;68:1417–29. doi:10.1136/gutjnl-2018-317609.
9. DanielH, GholamiAM, BerryD, DesmarchelierC, HahneH, LohG, et al. High-fat diet alters gut microbiota physiology in mice. ISME J. 2014;8:295–308. doi:10.1038/ismej.2013.155.

10. HeC, ChengD, PengC, LiY, ZhuY, LuN. High-Fat Diet Induces Dysbiosis of Gastric Microbiota Prior to Gut Microbiota in Association With Metabolic Disorders in Mice. Front Microbiol. 2018;9:639. doi:10.3389/fmicb.2018.00639.
11. SenderR, FuchsS, MiloR. Are We Really Vastly Outnumbered? Revisiting the Ratio of Bacterial to Host Cells in Humans. Cell. 2016;164:337–40.
12. MaruvadaP, LeoneV, KaplanLM, ChangEB. The Human Microbiome and Obesity: Moving beyond Associations. Cell Host Microbe. 2017;22:589–99.
13. JandhyalaSM, TalukdarR, SubramanyamC, VuyyuruH, SasikalaM, ReddyDN. Role of the normal gut microbiota. World J Gastroenterol. 2015;21:8787–803.
14. TomasJ, ReygnerJ, MayeurC, DucrocR, BouetS, BridonneauC, et al. Early colonizing Escherichia coli elicits remodeling of rat colonic epithelium shifting toward a new homeostatic state. ISME J. 2015;9:46–58.
15. CanforaEE, JockenJW, BlaakEE. Short-chain fatty acids in control of body weight and insulin sensitivity. Nat Rev Endocrinol. 2015;11:577–91.
16. ArumugamM, RaesJ, PelletierE, LePaslierD, YamadaT, MendeDR, et al. Enterotypes of the human gut microbiome. Nature. 2011;473:174–180.
17. TurnbaughPJ, LeyRE, MahowaldMA, MagriniV, MardisER, GordonJI. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature. 2006;444:1027–31.
18. TurnbaughPJ, BäckhedF, FultonL, GordonJI. Diet-Induced Obesity Is Linked to Marked but Reversible Alterations in the Mouse Distal Gut Microbiome. Cell Host Microbe. 2008;3:213–23.
19. LeyRE, TurnbaughPJ, KleinS, GordonJI. Microbial ecology: Human gut microbes associated with obesity. Nature. 2006;444:1022–3.
20. ShinNR, LeeJC, LeeHY, KimMS, WhonTW, LeeMS, et al. An increase in the Akkermansia spp. population induced by metformin treatment improves glucose homeostasis in diet-induced obese mice. Gut. 2014;63:727–35.
21. Moya-PérezA, NeefA, SanzY. Bifidobacterium pseudocatenulatum CECT 7765 reduces obesity-associated inflammation by restoring the lymphocyte-macrophage balance and gut microbiota structure in high-fat diet-fed mice. PLoS One. 2015;10:e0126976.
22. TurnbaughPJ, RidauraVK, FaithJJ, ReyFE, KnightR, GordonJI. The effect of diet on the human gut microbiome: A metagenomic analysis in humanized gnotobiotic mice. Sci Transl Med. 2009;1:6ra14.
23. Gauffin CanoP, SantacruzA, MoyaÁ, SanzY. Bacteroides uniformis CECT 7771 ameliorates metabolic and immunological dysfunction in mice with high-fat-diet induced obesity. PLoS One. 2012;7:e41079.
24. MurphyEA, VelazquezKT, HerbertKM. Influence of high-fat diet on gut microbiota: A driving force for chronic disease risk. Curr Opin Clin Nutr Metab Care. 2015;18:515–20. doi:10.1097/MCO.0000000000000209.

25. CaoY, ZouS, XuH, LiM, TongZ, XuM, et al. Hypoglycemic activity of the Baker's yeast β-glucan in obese/type 2 diabetic mice and the underlying mechanism. Mol Nutr Food Res. 2016;60:2678–90.
26. JiangT, GaoX, WuC, TianF, LeiQ, BiJ, et al. Apple-derived pectin modulates gut microbiota, improves gut barrier function, and attenuates metabolic endotoxemia in rats with diet-induced obesity. Nutrients. 2016;8:126.
27. O'KeefeSJ. The association between dietary fibre deficiency and high-income lifestyle-associated diseases: Burkitt's hypothesis revisited. Lancet Gastroenterol Hepatol. 2019;4:984–96.
28. MakkiK, DeehanEC, WalterJ, BäckhedF. The Impact of Dietary Fiber on Gut Microbiota in Host Health and Disease. Cell Host and Microbe. 2018;23:705–15.
29. CummingsJH, PomareEW, BranchHWJ, NaylorCPE, MacFarlaneGT. Short chain fatty acids in human large intestine, portal, hepatic and venous blood. Gut. 1987;28:1221–7.
30. MyhrstadMCW, TunsjøH, CharnockC, Telle-HansenVH. Dietary fiber, gut microbiota, and metabolic regulation—current status in human randomized trials. Nutrients. 2020;12.
31. ThenT, K.C, PaillasP, S.S, HampsonH, A.A, et al. The gut microbiota may drive the radiosensitising effect of a high fibre diet. bioRxiv Cancer Biol. 2020;:846436. doi:10.1101/846436.
32. YangJY, LeeYS, KimY, LeeSH, RyuS, FukudaS, et al. Gut commensal Bacteroides acidifaciens prevents obesity and improves insulin sensitivity in mice. Mucosal Immunol. 2017;10:104–16.
33. BeyazS, ManaMD, RoperJ, KedrinD, SaadatpourA, HongSJ, et al. High-fat diet enhances stemness and tumorigenicity of intestinal progenitors. Nature. 2016;531:53–8.
34. SchulzMD, AtayÇ, HeringerJ, RomrigFK, SchwitallaS, AydinB, et al. High-fat-diet-mediated dysbiosis promotes intestinal carcinogenesis independently of obesity. Nature. 2014;514:508–12.
35. GulhaneM, MurrayL, LourieR, TongH, ShengYH, WangR, et al. High Fat Diets Induce Colonic Epithelial Cell Stress and Inflammation that is Reversed by IL-22. Sci Rep. 2016;6:28990. doi:10.1038/srep28990.
36. DesaiMS, SeekatzAM, KoropatkinNM, KamadaN, HickeyCA, WolterM, et al. A Dietary Fiber-Deprived Gut Microbiota Degrades the Colonic Mucus Barrier and Enhances Pathogen Susceptibility. Cell. 2016;167:1339-1353.e21.
37. IjssennaggerN, BelzerC, HooiveldGJ, DekkerJ, VanMilSWC, MüllerM, et al. Gut microbiota facilitates dietary heme-induced epithelial hyperproliferation by opening the mucus barrier in colon. Proc Natl Acad Sci U S A. 2015;112:10038–43.
38. BoutardM, CerisyT, NoguePY, AlbertiA, WeissenbachJ, SalanoubatM, et al. Functional Diversity of Carbohydrate-Active Enzymes Enabling a Bacterium to Ferment Plant Biomass. PLoS Genet. 2014;10. doi:10.1371/journal.pgen.1004773.
39. LiH, LiuF, LuJ, ShiJ, GuanJ, YanF, et al. Probiotic Mixture of Lactobacillus plantarum Strains Improves Lipid Metabolism and Gut Microbiota Structure in High Fat Diet-Fed Mice. Front Microbiol. 2020;11:512. doi:10.3389/fmicb.2020.00512.

40. HuangS, PangD, LiX, YouL, ZhaoZ, CheungPCK, et al. A sulfated polysaccharide from: Gracilaria Lemaneiformis regulates cholesterol and bile acid metabolism in high-fat diet mice. Food Funct. 2019;10:3224–36.
41. HuS, WangJ, XuY, YangH, WangJ, XueC, et al. Anti-inflammation effects of fucosylated chondroitin sulphate from: Acaudina molpadioides by altering gut microbiota in obese mice. Food Funct. 2019;10:1736–46. doi:10.1039/c8fo02364f.
42. CuiHX, ZhangLS, LuoY, YuanK, HuangZY, GuoY. A purified anthraquinone-glycoside preparation from rhubarb ameliorates type 2 diabetes mellitus by modulating the gut microbiota and reducing inflammation. Front Microbiol. 2019;10 JUN:1423. doi:10.3389/fmicb.2019.01423.
43. LiuT, WangB, CaoH. Effects of high-fat diet-induced gut microbiota dysbiosis: Far beyond the gut. Gut. 2020.
44. HeymsfieldSB, PietrobelliA. Individual differences in apparent energy digestibility are larger than generally recognized. Am J Clin Nutr. 2011;94:1650–1. doi:10.3945/ajcn.111.026476.
45. LinH, AnY, HaoF, WangY, TangH. Correlations of Fecal Metabonomic and Microbiomic Changes Induced by High-fat Diet in the Pre-Obesity State. Sci Rep. 2016;6.
46. GurungM, LiZ, YouH, RodriguesR, JumpDB, MorgunA, et al. Role of gut microbiota in type 2 diabetes pathophysiology. EBioMedicine. 2020;51:102590. doi:10.1016/j.ebiom.2019.11.051.
47. KohA, DeVadderF, Kovatcheva-DatcharyP, BäckhedF. From dietary fiber to host physiology: Short-chain fatty acids as key bacterial metabolites. Cell. 2016;165:1332–45.
48. NakajimaA, SasakiT, ItohK, KitaharaT, TakemaY, HiramatsuK, et al. A soluble fiber diet increases Bacteroides fragilis group and IgA production in the gut. Appl Environ Microbiol. 2020.
49. PlovierH, EverardA, DruartC, DepommierC, VanHulM, GeurtsL, et al. A purified membrane protein from Akkermansia muciniphila or the pasteurized bacterium improves metabolism in obese and diabetic mice. Nat Med. 2017;23:107–13.
50. MatsakasA, ProsdocimoDA, MitchellR, Collins-HooperH, GiallourouN, SwannJR, et al. Investigating mechanisms underpinning the detrimental impact of a high-fat diet in the developing and adult hypermuscular myostatin null mouse. Skelet Muscle. 2015;5:38. doi:10.1186/s13395-015-0063-5.
51. BarneaM, ShamayA, StarkAH, MadarZ. A high-fat diet has a tissue-specific effect on adiponectin and related enzyme expression. Obesity. 2006;14:2145–53. doi:10.1038/oby.2006.251.
52. van derHeijdenRA, SheedfarF, MorrisonMC, HommelbergPPH, KorD, KloosterhuisNJ, et al. High-fat diet induced obesity primes inflammation in adipose tissue prior to liver in C57BL/6j mice. Aging (Albany NY). 2015;7:256–68. doi:10.18632/aging.100738.
53. RuedenCT, SchindelinJ, HinerMC, DeZoniaBE, WalterAE, ArenaET, et al. ImageJ2: ImageJ for the next generation of scientific image data. BMC Bioinformatics. 2017;18:529. doi:10.1186/s12859-017-1934-z.
54. MetsaluT, ViloJ. ClustVis: A web tool for visualizing clustering of multivariate data using Principal Component Analysis and heatmap. Nucleic Acids Res. 2015;43:W566–70. doi:10.1093/nar/gkv468.

55. SegataN, IzardJ, WaldronL, GeversD, MiropolskyL, GarrettWS, et al. Metagenomic biomarker discovery and explanation. Genome Biol. 2011;12.

Figures

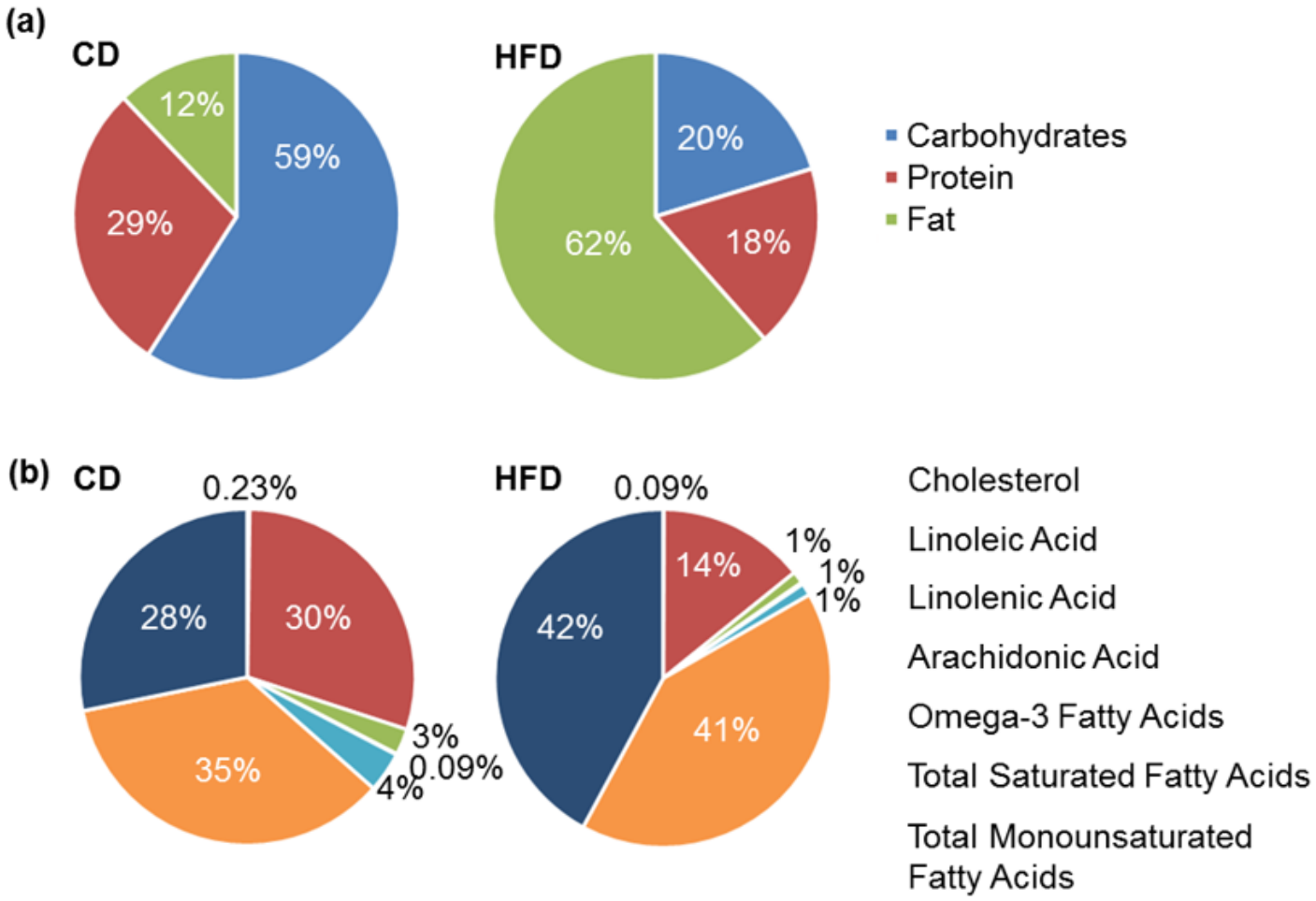

Figure 1

Composition of the HFD and CD in terms of (a) macronutrients providing calories and (b) fat content. HFD, high-fat diet; CD, chow diet.

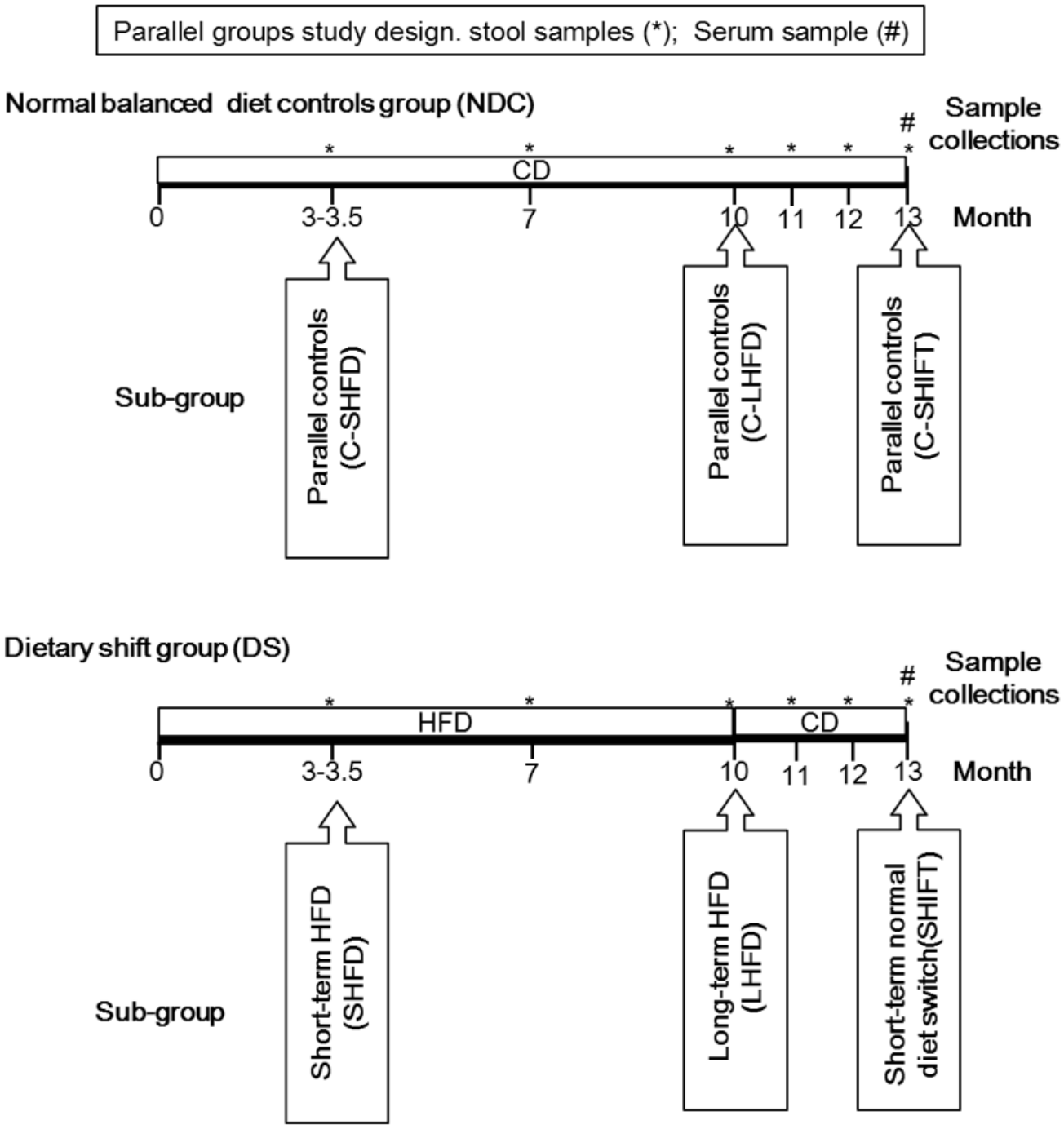

Figure 2

Schematic of the study's design illustrating the timeline of feeding strategies and sampling. Stool and serum sampling are marked as * and #, respectively. N = 3 in the normal balanced diet control group and N = 5 in the dietary shift group. HFD, high-fat diet; CD, chow diet; SHFD, short-term HFD; LHFD, long-term HFD.

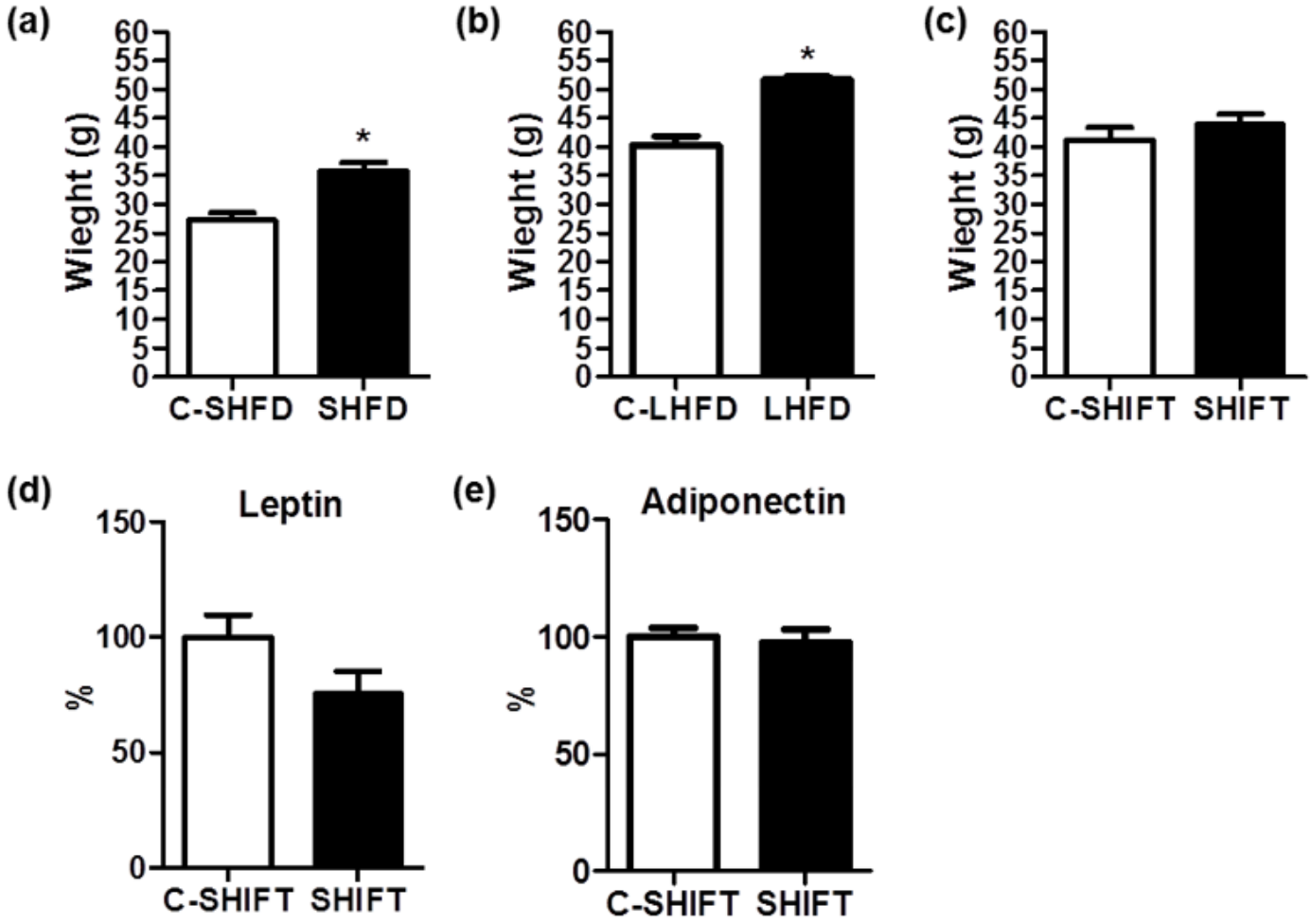

Figure 3

HFD-induced obesity was ameliorated by the dietary switch. Body weight in mice (a) fed with an SHFD (b), fed with an LHFD, and (c) that shifted from an HFD to a CD compared with their parallel controls. Serum (d) leptin and (e) adiponectin levels in mice following the dietary shift. *: $p < 0.05$ versus individual control group. N = 3 in the normal balanced diet control group and N = 5 in the dietary shift group. SHFD, short-term HFD; LHFD, long-term HFD.

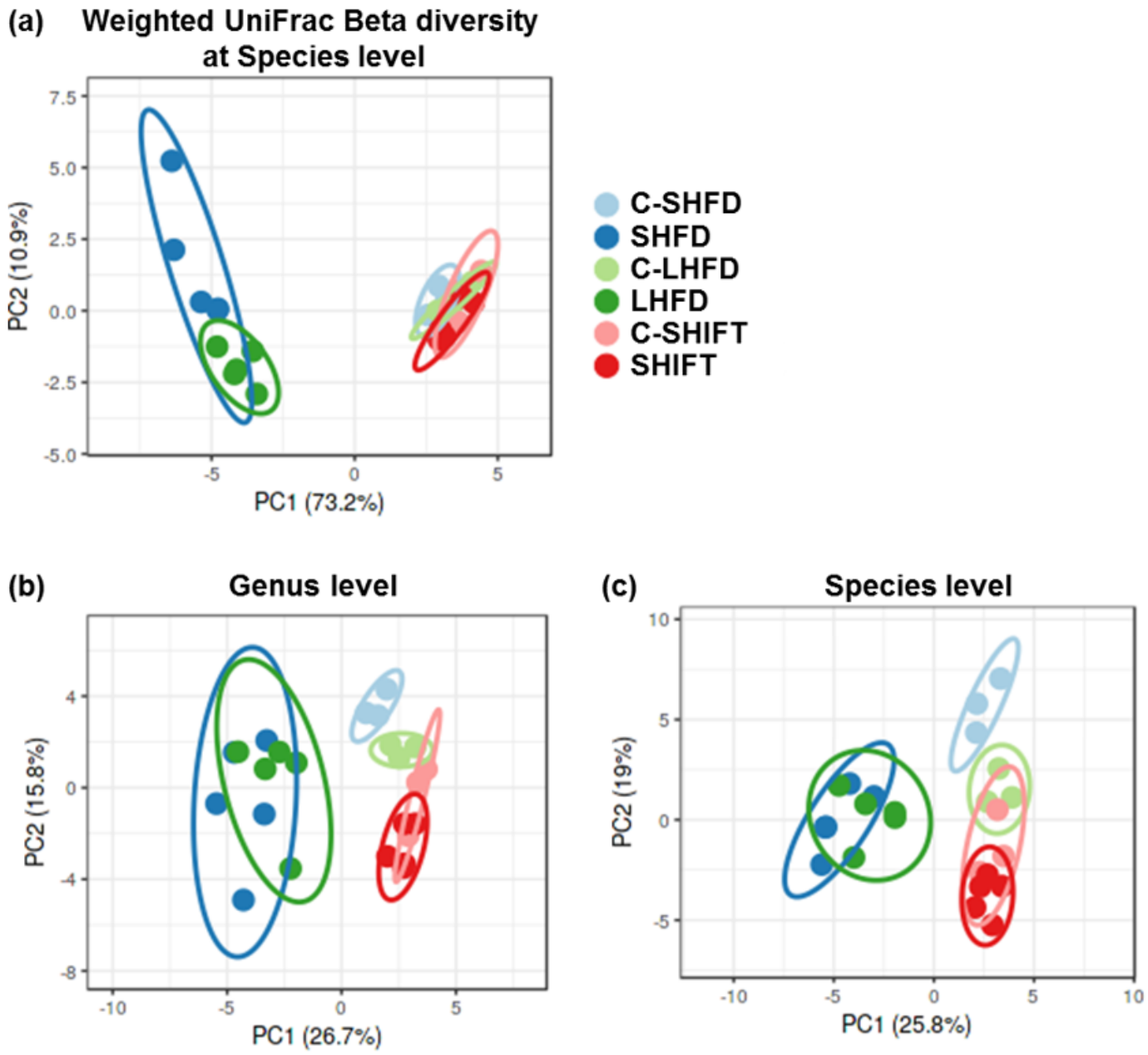

Figure 4

Principal component analysis chart. (a) Beta diversity at the species level according to weighted UniFrac analysis. Data were analyzed using ClustVis (b) at the genus level and (c) at the species level. SHFD: HFD feeding for 3 months; C-SHFD: the parallel controls of the SHFD group; LHFD: HFD feeding for 10 months; C-LHFD: the parallel controls of the LHFD group; SHIFT; dietary shift to a CD for 3 months after HFD feeding for 10 months; C-SHIFT: the parallel controls of the SHIFT group. N = 3 in normal balanced diet control group and N = 5 in the dietary shift group. HFD, high-fat diet; CD, chow diet; SHFD, short-term HFD; LHFD, long-term HFD.

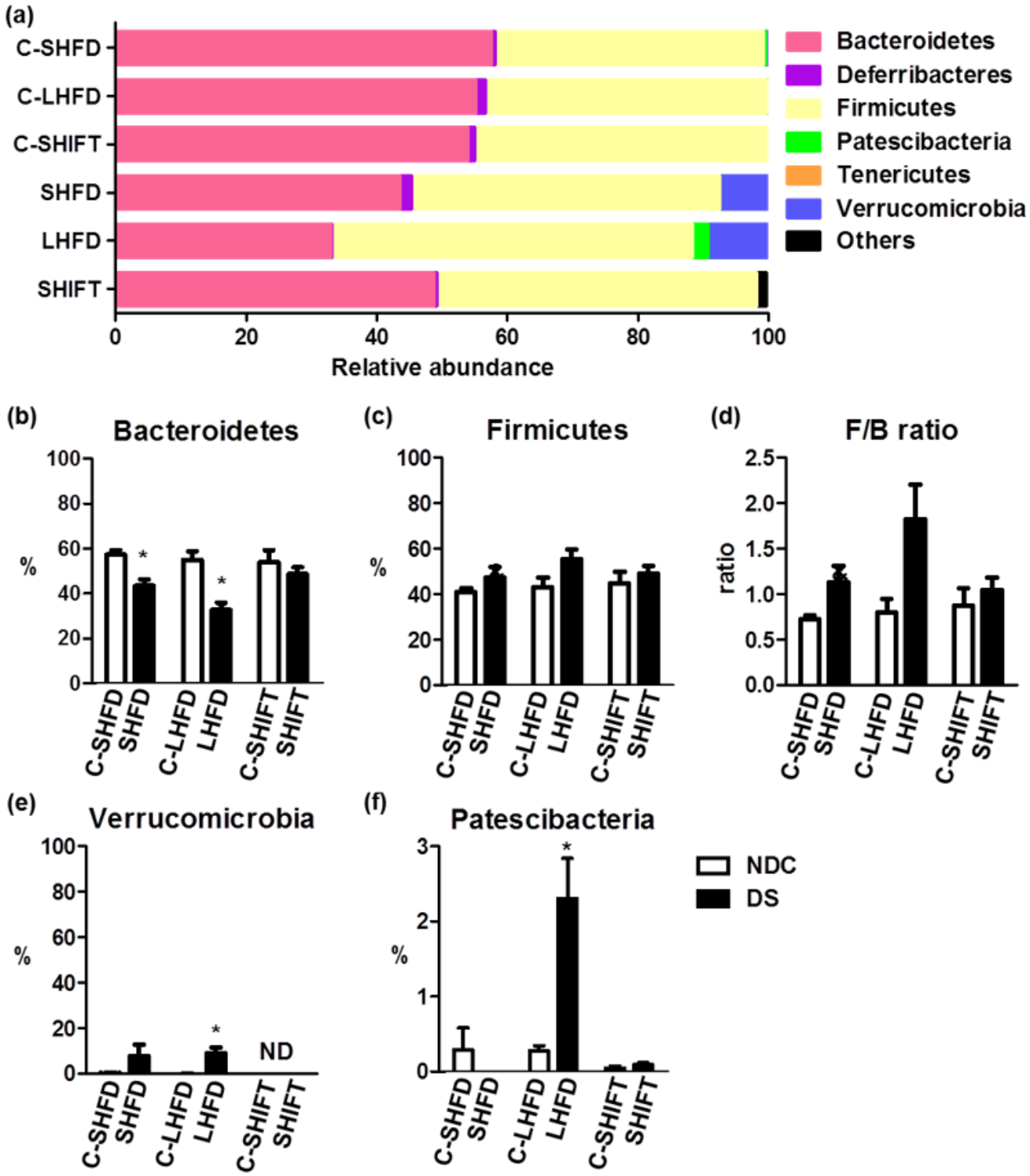

Figure 5

Effects of the dietary shift on the relative abundance of gut microbiota at the phylum level. (a) Composition. (b) The abundance of Bacteroidetes. (c) The abundance of Firmicutes. (d) The abundance of the F/B ratio. (e) The abundance of Verrucomicrobia. (f) The abundance of Patescibacteria. SHFD: HFD feeding for 3 months; C-SHFD: the parallel controls of the SHFD group; LHFD: HFD feeding for 10 months; C-LHFD: the parallel controls of the LHFD group; SHIFT; dietary shift to a CD for 3 months after

HFD feeding for 10 months; C-SHIFT: the parallel controls of the SHIFT group. *: $p < 0.05$ versus the individual control group. N = 3 in the NDC group and N = 5 in the DS group. HFD, high-fat diet; CD, chow diet; SHFD, short-term HFD; LHFD, long-term HFD; NDC, normal balanced diet control; DS, dietary shifting.

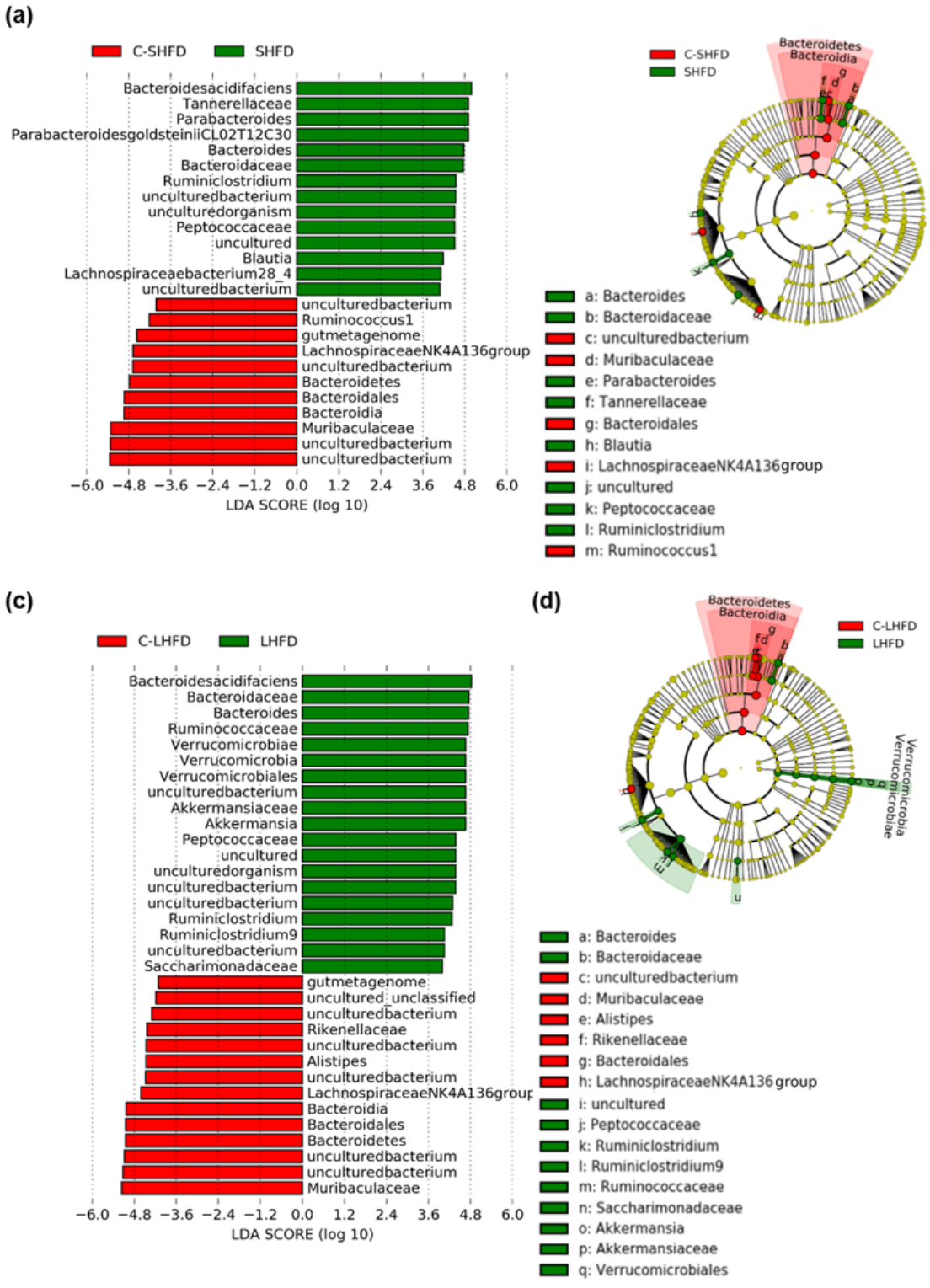

Figure 6

LEfSe analysis. (a, c) LDA score at the SHFD and LHFD, , respectively. (b, d) Cladogram at the SHFD and LHFD, respectively. The LDA significant thresholds were 4.0 at SHFD and LHFD stages. SHFD: HFD

feeding for 3 months; C-SHFD: the parallel controls of the SHFD group; LHFD: HFD feeding for 10 months; C-LHFD: the parallel controls of the LHFD group. N = 3 in normal balanced diet control group and N = 5 in the dietary shift group. HFD, high-fat diet; CD, chow diet; SHFD, short-term HFD; LHFD, long-term HFD.

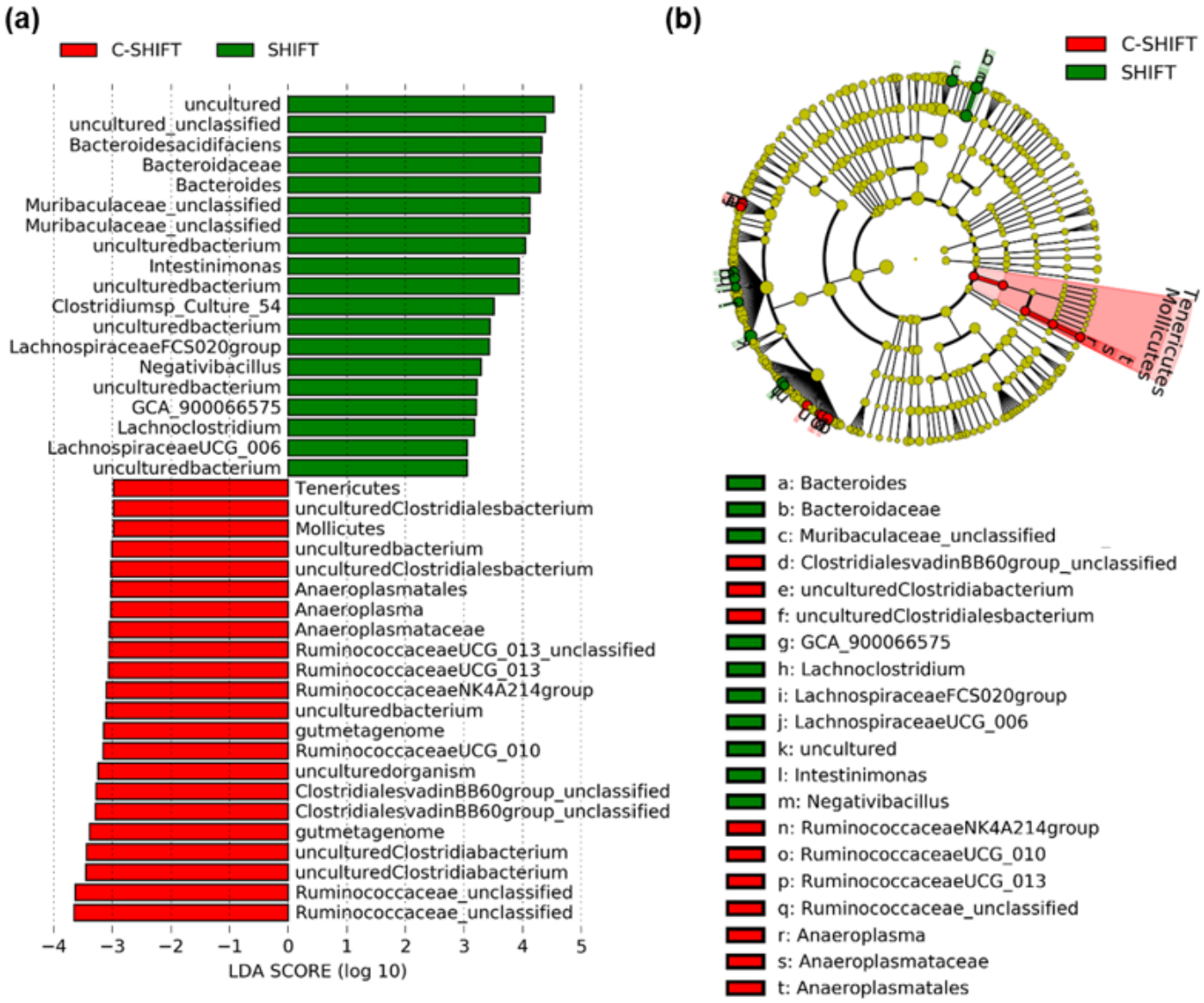

Figure 7

LEfSe analysis. (a) LDA score at the SHIFT stages. (b) Cladogram at the SHIFT stages. The LDA significant thresholdwas 2.0 at SHIFT stage. SHIFT; dietary shift to a CD for 3 months after HFD feeding for 10 months; C-SHIFT: the parallel controls of the SHIFT group. N = 3 in normal balanced diet control group and N = 5 in the dietary shift group..

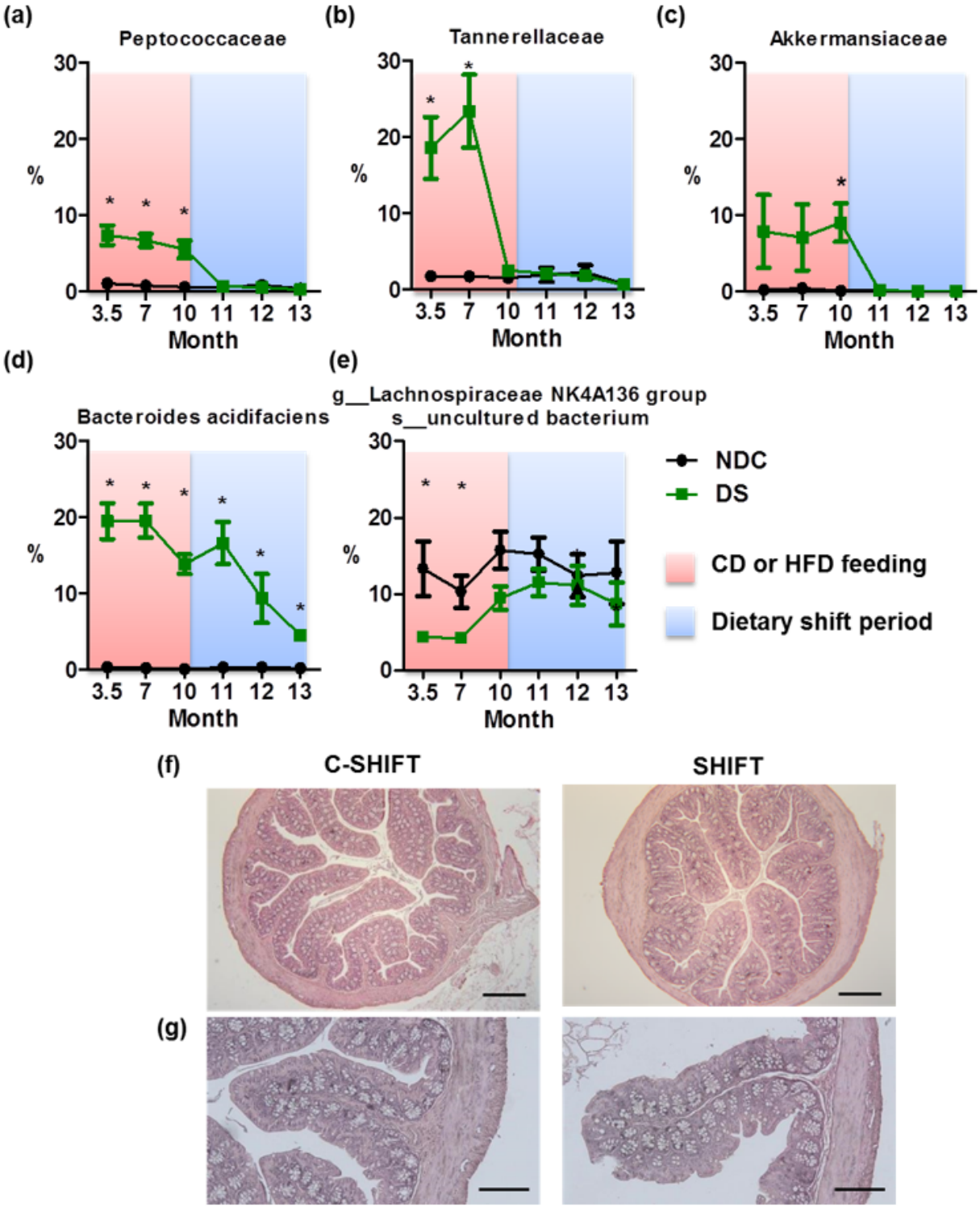

Figure 8

Effects of the dietary shift on the relative abundance of gut microbiota at the family level. (a) Peptococcaceae. (b) Tannerellaceae. (c) Akkermansiaceae. At the genus level: (d) Bacteroides acidifaciens. (e) uncultured bacterium belonging to Lachnospiraceae NK4A136 group. (f) H&E stained colon sections at low magnification (scale bar: 400 μm). (g) H&E stained colon sections at high magnification (scale bar: 50 μm). Red and blue backgrounds indicate HFD feeding and dietary shift to CD,

respectively. *: $p < 0.05$ versus the individual control group. SHIFT; dietary shift to a CD for 3 months after HFD feeding for 10 months; C-SHIFT: the parallel controls of the SHIFT group. N = 3 in NDC group and N = 5 in DS group. HFD, high-fat diet; CD, chow diet; NDC, normal balanced diet control; DS, dietary shifting; H&E, hematoxylin and eosin.

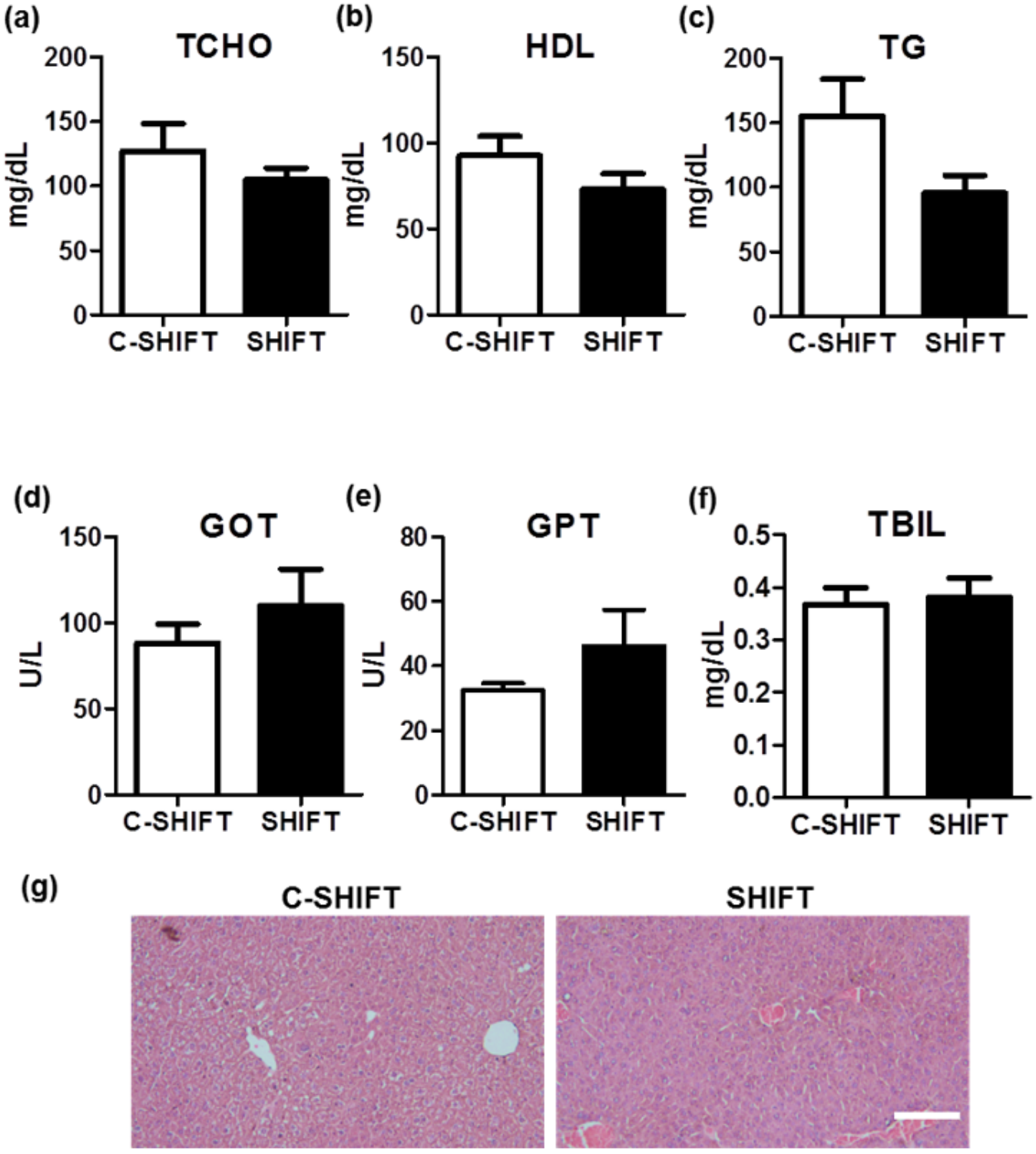

Figure 9

No difference in blood lipid profiles and hepatic function parameters of mice that underwent the dietary shift. Serum levels of (a) TCHO, (b) HDL, and (c) LDL) were measured. Serum levels of (d) GOT, (e) GPT, and (f) TBIL (g) hematoxylin and eosin–stained liver sections (scale bar: 50 μm). SHIFT: dietary shift to a CD for 3 months after HFD feeding for 10 months; C-SHIFT: the parallel controls of the SHIFT group. TCHO, total cholesterol; HDL, high-density lipoproteins; LDL, low-density lipoproteins; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; TBIL, total bilirubin; HFD, high-fat diet; CD, chow diet.

Supplementary Files

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

- AuthorChecklistFull202007293.pdf
- additionalfile2.csv
- Addtionalfile1.pptx