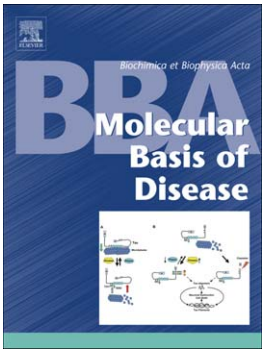


Arachidonic acid sex-dependently affects obesity through linking gut microbiota-driven inflammation to hypothalamus-adipose-liver axis

Pan Zhuang, Qiyang Shou, Yanhua Lu, Guangfa Wang, Jieni Qiu, Jun Wang, Lilin He, Jingnan Chen, Jingjing Jiao, Yu Zhang

PII: S0925-4439(17)30227-2
DOI: doi:10.1016/j.bbadis.2017.07.003
Reference: BBADIS 64815

To appear in: *BBA - Molecular Basis of Disease*

Please cite this article as: Pan Zhuang, Qiyang Shou, Yanhua Lu, Guangfa Wang, Jieni Qiu, Jun Wang, Lilin He, Jingnan Chen, Jingjing Jiao, Yu Zhang, Arachidonic acid sex-dependently affects obesity through linking gut microbiota-driven inflammation to hypothalamus-adipose-liver axis, *BBA - Molecular Basis of Disease* (2017), doi:10.1016/j.bbadis.2017.07.003

Arachidonic acid sex-dependently affects obesity through linking gut microbiota-driven inflammation to hypothalamus-adipose-liver axis

Pan Zhuang [b], Qiyang Shou [c], Yanhua Lu [d], Guangfa Wang [e], Jieni Qiu [a], Jun Wang [b], Lilin He [b], Jingnan Chen [b], Jingjing Jiao [a,*], Yu Zhang [b,*]

[a] *Department of Nutrition, School of Public Health, Zhejiang University School of Medicine, Hangzhou 310058, Zhejiang, China*

[b] *National Engineering Laboratory of Intelligent Food Technology and Equipment, Zhejiang Key Laboratory for Agro-Food Processing; Fuli Institute of Food Science, College of Biosystems Engineering and Food Science, Zhejiang University, Hangzhou 310058, Zhejiang, China*

[c] *Experimental Animal Research Center & Institute of Comparative Medicine, Zhejiang Chinese Medical University, Hangzhou 310053, Zhejiang, China*

[d] *Institute of Aging Research, Hangzhou Normal University School of Medicine, Hangzhou 311121, Zhejiang, China*

[e] *Department of PET Center, The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou 310003, Zhejiang, China*

* Corresponding authors

E-mail address: y_zhang@zju.edu.cn (Y. Zhang), jingjingjiao@zju.edu.cn (J. Jiao).

ABSTRACT

Unraveling the role of dietary lipids is beneficial to treat obesity and metabolic dysfunction. Nonetheless, how dietary lipids affect existing obesity remains unknown. Arachidonic acid (AA), a derivative of linoleic acid, is one of the crucial n-6 fatty acids. The aim of this study was to investigate whether AA affects obesity through associating microbiota-driven inflammation with hypothalamus-adipose-liver axis. Four-week old C57BL/6J mice were fed with a high-fat diet (HFD, 45% fat) for 10 weeks to induce obesity, and then fed a HFD enriched with 10 g/kg of AA or a continuous HFD in the following 15 weeks. Systemic adiposity and inflammation, metabolic profiles, gut microbiota composition, short-chain fatty acids production, hypothalamic feeding regulators, browning process of adipocytes, hepatosteatosis, and insulin resistance in adipose were investigated. The results indicated that AA aggravates obesity for both genders whereas sex-dependently affects gut microbiota composition. Also, AA favors pro-inflammatory microbiota and reduces butyrate production and circulating serotonin, which augments global inflammation and triggers hypothalamic leptin resistance via microglia accumulation in male. AA exacerbates non-alcoholic steatohepatitis along with amplified inflammation through TLR4-NF-κB pathway and induces insulin resistance. Reversely, AA alleviates obesity-related disorders via rescuing anti-inflammatory and butyrate-producing microbiota, up-regulating *GPR41* and *GPR109A* and controlling hypothalamic inflammation in female. Nevertheless, AA modifies adipocyte browning and promotes lipid mobilization for both genders. We show that AA affects obesity likely through a gut-hypothalamus-adipose-liver axis. Our findings formulate recommendations of n-6 fatty acids like AA from dietary intake for obese subjects preferably in a sexually dimorphic way.

Keywords: arachidonic acid; obesity; sexual dimorphism; gut microbiota; butyrate; hypothalamus-adipose-liver axis

1. Introduction

Obesity is a major risk factor for a cluster of systemic and chronic disorders, including type 2 diabetes (T2D) [1] and non-alcoholic fatty liver disease (NAFLD) [2]. Whilst high-fat diet (HFD) is generally considered obesogenic, the profile of dietary fatty acids becomes a critical factor concerning diet-induced obesity (DIO). However, what less well understood is the effect of lipids on developed obesity. Given the increasing consumption of n-6 polyunsaturated fatty acids (PUFA) especially in Western countries, it is important to assess potential impact of dietary n-6 PUFA on obese subjects.

Elevated markers of inflammation are interrelated with an increased risk of obesity-related metabolic diseases including T2D [3,4]. DIO causes metabolic inflammation in the mediobasal hypothalamus critical for energy homeostasis [5]. This process is mediated by microglia accumulation and activation, which leads to leptin resistance and consequently promotes appetite and food intake [6]. Meanwhile, the development of obesity-associated metabolic complications is sexually dimorphic. Premenopausal women are protected from the detrimental effects of obesity due to 17β-estradiol and estrogen receptor α (ERα) [7,8]. HFD is usually proposed to promote inflammation via hypothalamic regulation of PGC1α and ERα in a sex-specific way [9]. However, it is unknown whether n-6 PUFA intake sex-dependently affects inflammation and obesity-related disease.

Alterations of gut microbial composition are linked with obesity and related metabolic syndromes [10]. Obesity-induced gut dysbiosis impairs intestinal integrity, which releases the endotoxin lipopolysaccaride (LPS) from gut microbiota into the bloodstream [11]. This influx of LPS, in turn, activates *TLR4*-dependent signaling, leading to inflammation and insulin resistance (IR) [12]. In addition, the microbial metabolic byproducts short-chain fatty acids (SCFA) are recognized as essential sources of host energy and benefit the energy metabolism, which act as signaling molecules via G-protein coupled receptors (GPR) [13]. SCFA and other microbial metabolites also affect the production of serotonin (5-HT), which prolongs satiety

and regulates various physiological functions [14]. Collectively, gut microbiota may play a critical role in regulating host metabolism via the interplays among gut, brain, adipose and liver [15,16]. Dietary n-6 PUFA induce bacterial overgrowth by increasing intestinal Proteobacteria and dysbiosis. Nonetheless, since researches on microbial shifts by n-6 PUFA mainly focus on linoleic acid (LA) rather than arachidonic acid (AA), the impact of the latter still remains unknown.

AA, a derivative of LA, is one of the crucial n-6 fatty acids. The metabolites of AA include series of prostaglandins and leukotrienes, which are highly-active inflammatory mediators [17]. Herein, we explored the systemic effect of a long-term AA treatment on gut microbiota composition, inflammation, lipid, glucose and energy homeostasis in DIO mice, and importantly, the sexual dimorphisms.

2. Materials and methods

2.1. Animals

All animal experiments were approved by the Institutional Animal Care and Use Committee of Zhejiang University School of Medicine. Four-week old C57BL/6J mice (18±1 g, SPF, Laboratory Animal Research Center, Zhejiang Chinese Medical University, Hangzhou, China) were kept in 12-h day/night cycles with free access to water and fed with a high-fat diet (HFD, 45% fat) (Medicience Ltd., Yangzhou, Jiangsu, China) for 10 weeks to induce obesity and a low-fat diet (LFD, 10% fat) (Medicience) was used as a control diet (Supplementary Table 1). The HFD fed mice were then divided into two groups according to body weights and serum parameters, and fed a HFD enriched with 10 g/kg of arachidonic acid (AA, Nu-Chek Prep, Inc., Elysian, MN, USA) or a HFD in the following 15 weeks to investigate the effect of AA on exsiting obesity and related disorders. At the end of the dietary intervention, fasted mice were anaesthetized and killed by cervical dislocation, and blood samples were then collected. Tissues were harvested and weighed, and then snap-frozen in liquid nitrogen and stored at -80°C until further use. Caecal content was also collected for gut microbiota and SCFA analysis by the aseptic technique. Oral glucose tolerance

test (OGTT) and insulin tolerance test (ITT) were measured prior to sacrifice and described in details in Supplementary Methods.

2.2. Indirect Calorimetry and Micro-Positron Emission Tomography/Computed Tomography (Micro-PET/CT)

Metabolic parameters were measured at week 24 by an 8-cage animal monitoring system (TSE PhenoMaster/LabMaster, Bad Homburg, Germany). Brown adipose tissue (BAT) imaging was conducted using a small animal-dedicated micro-PET/CT system (Siemens Preclinical Solutions, Knoxville, TN, USA). Detailed procedures are described in Supplementary Methods.

2.3. Biochemical Analyses

A 7020 automatic biochemistry analyzer (Hitachi Ltd., Tokyo, Japan) was used to determine serum concentrations of glucose, triglyceride (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C). Glycosylated hemoglobin in whole blood was determined by routine ionic exchange high performance liquid chromatography (HPLC) method. Serum levels of adiponectin, leptin, insulin, serotonin, lipopolysaccaride (LPS), interleukin-6 (IL6) and tumor necrosis factor α (TNFα) were detected with commercial enzyme linked immunosorbent assay (ELISA) kits (Cusabio Biotech Co. Ltd., Wuhan, Hubei, China). Liver TG were isolated and quantified using a colorimetric assay kit (Applygen, Beijing, China) according to the manufacturer's instructions. Cholesterols including HDL-C, LDL-C and very low density lipoprotein cholesterol (VLDL-C) were assessed using commercial ELISA kits (Cusabio Biotech).

2.4. Real-Time PCR

Gene expression analysis by RT-PCR was performed in adipose tissues, liver and hypothalamus. Hepatic miRNA expression was also analyzed. Methodologies are detailed in Supplementary Methods.

2.5. Western Blot Analysis

Total cellular protein from liver, hypothalamus and adipose tissues was extracted and analyzed using SDS-PAGE and immunoblots. Mitochondrial proteins were extracted from white adipose tissue (WAT) and also analyzed by Western blot. Detailed protocols and antibodies used are described in Supplementary Methods. Quantitative results of Western blot analysis were shown in Supplementary Fig. 1.

2.6. Morphometric Analysis and Immunohistochemistry

Freshly isolated tissues were fixed overnight in 4% formaldehyde, then dehydrated and embedded in paraffin. Portions of 3-μm tissue sections were stained with haematoxylin and eosin (H-E) following standard protocols and adipose tissue sections were stained with UCP1. Images were analyzed using the Image J software (U.S. National Institutes of Health, Bethesda, MD, USA).

2.7. Immunofluorescence

Brains and adipose tissues were embedded in optimal cutting temperature and then cut into sections. Brains were stained for Iba1 and WAT were double-stained for F4/80 and CD11c. Staining protocols and image analysis are detailed in Supplementary Methods.

2.8. Gut Microbiota and SCFA Concentration Analysis

DNA was extracted from caecal content samples followed by PCR amplification and 16S rDNA sequencing. SCFA was also extracted from caecal content and quantified by gas chromatography (GC) analysis. Detailed procedures for gut microbiota and SCFA anaylses are shown in Supplementary Methods.

2.9. Statistical Analysis

Data were represented as mean ± SEM. Differences between two groups were

analyzed by two-tailed Student's *t* test. One-way analysis of variance (ANOVA) was applied to assess the statistical significance of more than two groups and two-way ANOVA was used for data from indirect calorimetry experiments. $P<0.05$ was considered statistically significant.

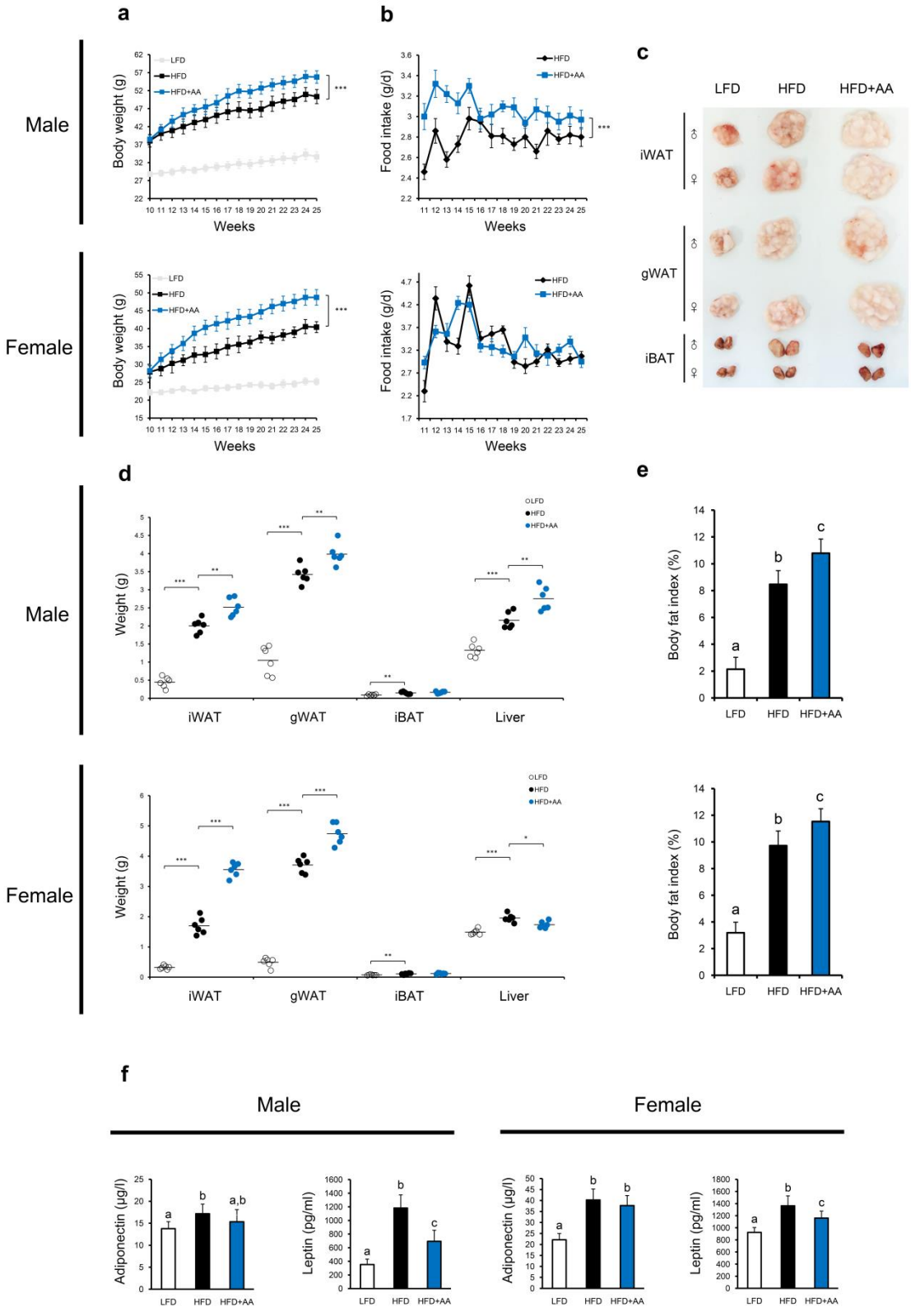


Fig. 1. AA aggravates obesity in DIO mice. (*n*=6). (**a**) Body weight curves. (**b**) Food intake. (**c**) Representative images of WAT and BAT. (**d**) Adipose tissue and liver weights. (**e**) Body fat index (%), ratio of retroperitoneal and gonadal fat weights to

body weight. (**f**) Serum levels of adiponectin and leptin. **P*<0.05, ***P*<0.01, ****P*<0.001, two-way ANOVA (**a,b**); Student's *t*-test (**d**); one-way ANOVA (**e,f**). Graph bars with different superscript letters appear significantly different (*P*<0.05).

3. Results

3.1. AA aggravates obesity in HFD-fed obese mice

To assess the effect of AA on DIO, we fed mice with a 10-week HFD to introduce obesity (Supplementary Fig. 2). HFD supplemented with AA (HFD+AA; 10 g/kg AA) was then served during the following 15 weeks. Finally, body weight of AA-receiving mice increased by 10.8% for male and 20.6% for female, respectively (Fig. 1a). Additionally, HFD+AA fed mice had significantly higher body fat index with increased gonadal and inguinal fat accumulation than HFD-fed mice (Fig. 1c,e).

Circulating leptin level was significantly reduced and a trend towards decreased serum adiponectin level was detected in AA-fed DIO mice (Fig. 1f). Although supplementation with AA elevated liver weight by 43.2% and food intake in male mice, such effects were not observed in female (Fig. 1b,d).

3.2. AA alters gut microbiota composition

Profiles of the gut microbiota composition in HFD+AA fed mice measured by high-throughput sequencing showed dramatic changes in a sex-specific manner. Principal coordinate analysis (PCoA) of the unweighted UniFrac demonstrated a distinct clustering of samples according to treatment (Fig. 2a,3a). As expected, microbial community diversity indicated by chao1 decreased in samples from HFD-fed mice compared with LFD-fed mice, while AA-fed DIO mice also showed a significant decrease in microbial richness (Fig. 2b,3b). Comparison of proportional abundance in phylum level showed that treatment with AA reduced the Firmicutes/Bacteroidetes ratio (Fig. 2c,3c). Notably, we observed significantly increased and decreased levels of Proteobacteria and Verrucomicrobia phylum only in HFD+AA fed female mice (Fig. 3c). These results suggest that AA exacerbates the

dysbiosis of intestinal flora in HFD-fed mice.

Male

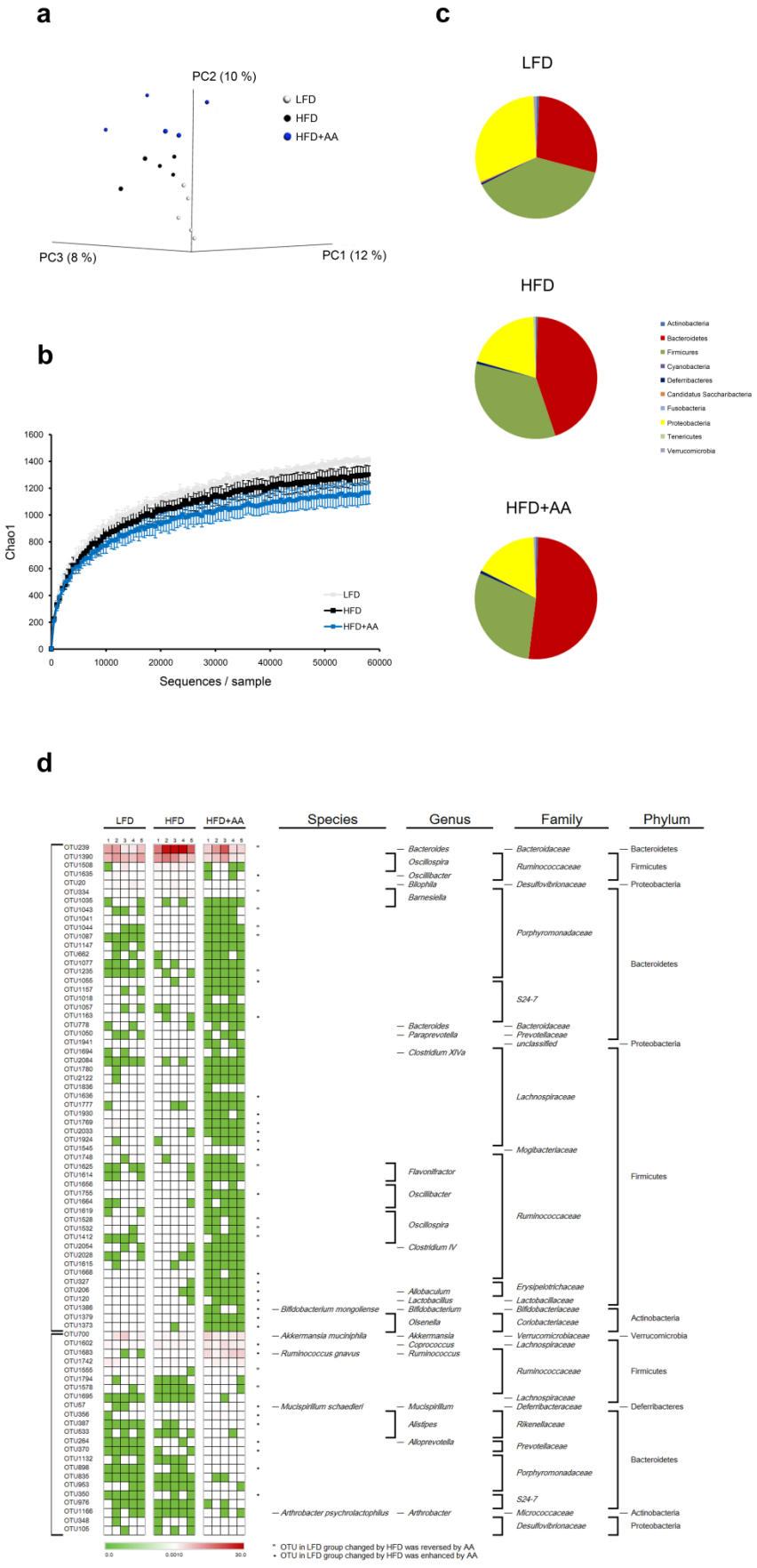


Fig. 2. AA alters gut microbiota composition in male mice (*n*=5). (**a**) PCoA analysis (unweighted UniFrac). (**b**) Rarefaction curves for Chao1 (1-58001 sequences per sample). (**c**) Pie charts of intestinal microbial composition at the phylum level. (**d**) Heatmaps based on RDA.

Female

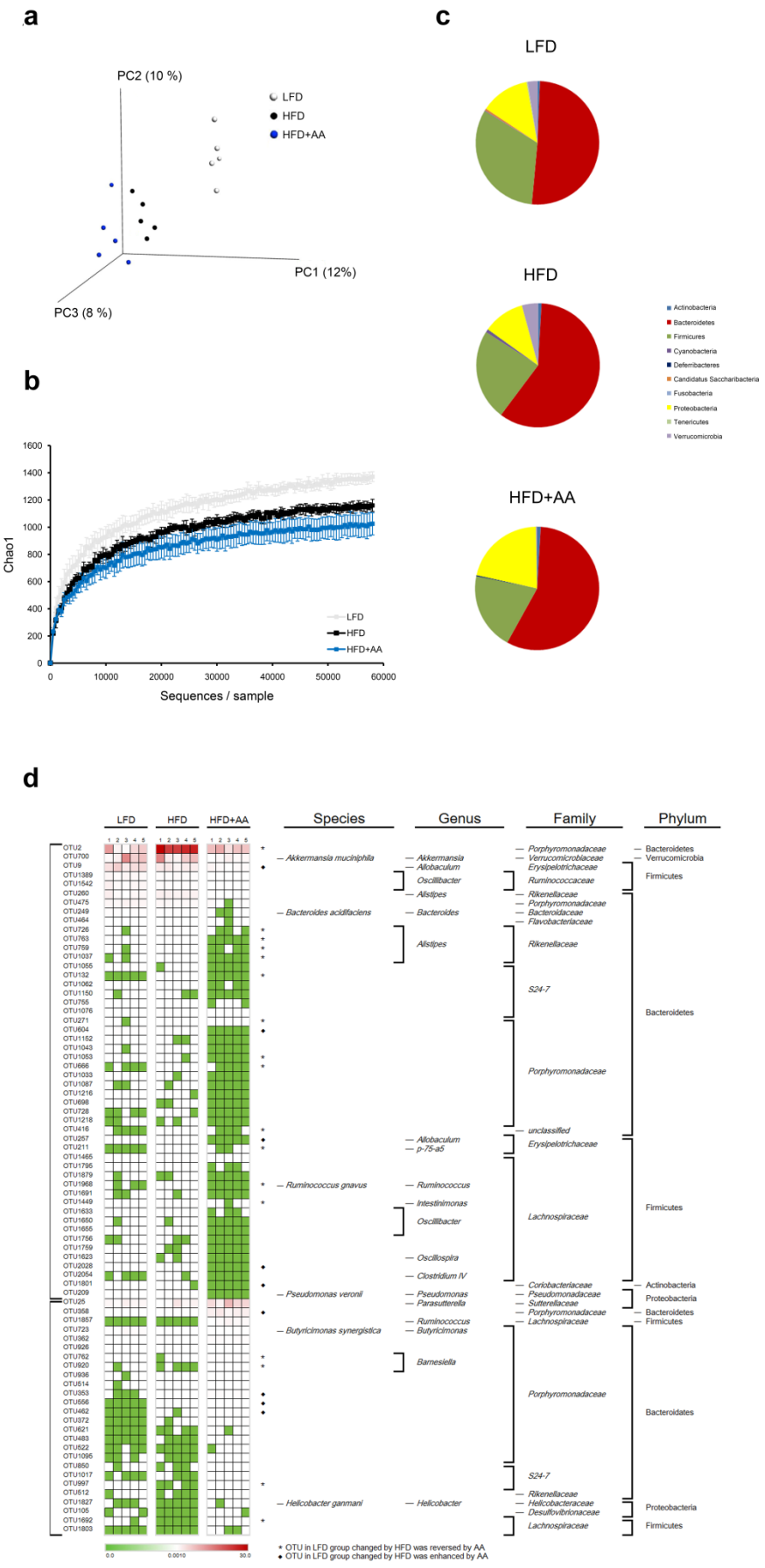


Fig. 3. AA alters gut microbiota composition in female mice (*n*=5). (**a**) PCoA analysis (unweighted UniFrac). (**b**) Rarefaction curves for Chao1. (**c**) Pie charts of intestinal microbial composition at the phylum level. (**d**) Heatmaps based on RDA.

AA exacerbates the dysbiosis of intestinal flora in HFD-fed mice. We further applied redundancy analysis (RDA) to distinguish the significantly altered bacterial phylotypes among the detected 2163 operational taxonomic units (OTUs). Compared with HFD fed mice, HFD supplemented with AA significantly altered 78 OTUs (55 decreased and 23 increased within 17 families) and 76 OTUs (50 decreased and 26 increased within 16 families) in male and female mice, respectively (Fig. 2d,3d). As opposed to HFD-fed mice, HFD+AA fed male mice had less abundant bacterial species from genera *Allobaculum*, *Oscillibacter*, *Bacteroides*, *Lactobacillus*, *Bifidobacterium*, which were initially found to be reduced by HFD and negatively associated with obesity, and more abundant species *Ruminococcus gnavus*, *Mucispirillum schaedleri* and genera *Coprococc*us, *Alistipes*, which were enhanced by HFD and positively associated with obesity (Fig. 2d). Moreover, AA also decreased the growth of *Oscillospira* and *Clostridium* XlVa and IV negatively related to obesity in male mice. Notably, *Akkermansia muciniphila* associated with inflammation and IR was found to be the most enriched bacteria in HFD+AA fed male mice. However, in female, despite similar decrease in *Allobaculum*, *Oscillibacter*, *Bacteroides acidifaciens*, *Oscillospira* and *Clostridium* IV, we observed increase in *Barnesiella*, which was initially reduced by HFD and recognized as anti-inflammatory properties, and decrease in *Alistipes* and *Ruminococcus gnavus*, which was enriched by HFD and positively correlated with inflammation. Additionally, *Pseudomonas veronii* associated with intestinal inflammatory pseudotumors and *Akkermansia muciniphila* were also reduced by AA in female mice. Taken together, AA treatment exerts a profound sex-dependent effect on gut microbiota composition in DIO mice. The shifts of inflammation/obesity-correlated microbiota seem negative for male mice. In contrast, AA protected several anti-inflammatory bacteria and reversed

pro-inflammatory microbiota initially altered by HFD in female mice which may help counteract inflammation.

HFD feeding increased circulating level of LPS and decreased villus and crypts length along with thinning of mucosal thickness depicted in cecum sections. Supplementation of AA augmented these changes in both genders (Supplementary Fig. 3a,b). To further investigate whether AA also affects gut microbial metabolites production, we next analyzed the concentration of SCFA in caecal contents using gas chromatography. Although total SCFA levels were not affected, we observed elevated acetate and reduced butyrate levels in HFD+AA fed male mice but opposite results in female mice (Supplementary Fig. 3d). This was correlated with the increase in butyrate-producing bacteria (*Butyricimonas synergistica* and *Ruminococcus*) in AA-treated female mice (Fig. 3d). SCFA have been identified as endogenous ligands for GPR, specifically *GPR43* showing the highest affinity for acetate while *GPR41* and *GPR109A* exhibiting the affinity for butyrate. In line with the changes in SCFA, we observed increased mRNA expression of *GPR43* in adipose tissue and liver of AA-treated male mice, and induction of *GPR41* expression in the two tissues and *GRP109A* expression in hypothalamus in female (Supplementary Fig. 3e). Also interestingly, serum serotonin level increased by HFD was reduced by AA in male but was promoted in female (Supplementary Fig. 3c).

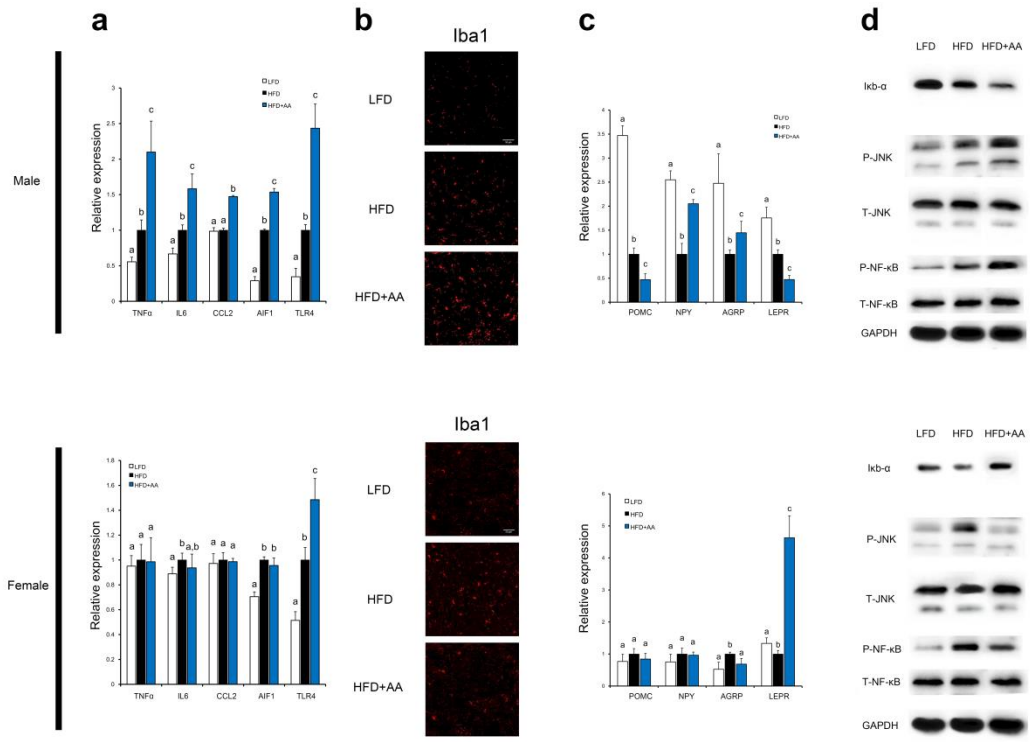


Fig. 4. AA augments hypothalamic inflammation and promotes NF-κB signaling (*n*=6). (**a**) Relative expression of inflammation markers and *TLR4*. (**b**) Iba1 staining in the ARC. Scale bar, 50 μm. (**c**) Relative expression of leptin-related neural peptides. (**d**) Representative hypothalamus immunoblots. The corresponding densitometry measurements were shown in Supplemental Fig. 1. Graph bars with different superscript letters appear significantly different ($P<0.05$).

3.3. AA affects inflammation in the hypothalamus

Accumulating evidence has uncovered the compelling crosstalk between gut and brain [15]. Thus, we next investigate whether AA also impacts neuroinflammation in the hypothalamus. As expected, hypothalamic mRNA levels of *TNF-α*, *IL6* and *CCl2* were significantly increased, which were also co-localized with the accumulation of microglia indicated by increased *Aif1/Iba1* mRNA expression via immunostaining in the arcuate nucleus (ARC) of AA-treated male mice. However, we did not significantly detect any change in female (Fig. 4a,b). Furthermore, we found that supplementation with AA elevated mRNA expression of agouti-related peptide (*AGRP*) and neuropeptide Y (*NPY*) whereas reduced expression of

pro-opiomelanocortin (*POMC*) and leptin receptor (*LEPR*) in male mice (Fig. 4c), which agree with the hyperphagia phenotype (Fig. 1b). However, decreased *AGRP* mRNA level and no significant alterations in expression of *NPY* and *POMC* were observed while the expression of *LEPR* and *GRP109A* was significantly up-regulated in AA-treated female mice (Fig. 4c; Supplementary Fig. 3e). Additionally, AA enhanced activation of phosphorylated JNK and inhibited production of IκB-α, leading to elevated NF-κB phosphorylation in DIO male mice, but reversely functioned in female (Fig. 4d). These results indicated that AA may reduce leptin sensitivity and impair leptin signaling in central melanocortin system via NF-κB signaling pathway in DIO male rather than female mice.

3.4. AA modifies browning process of adipocytes

Gut interacts with adipose to orchestrate energy homeostasis [18]. To investigate whether the thermogenesis of BAT was affected, we performed micro-PET/CT to assess [^{18}F]fluorodeoxyglucose (FDG) uptake of interscapular BAT (iBAT). As shown in Supplementary Fig. 4a, FDG uptake was lower in HFD group compared with LFD group and AA further significantly lowered FDG uptake in both HFD+AA fed male and female mice. In accordance with decreased metabolic activity of iBAT, AA treatment reduced *UCP1* level as revealed by immunohistochemistry (Supplementary Fig. 4b). In addition, we detected suppressions of the browning programme markers (*UCP1*, *PRDM16*, *BMP7*, *CEBPβ* and *PGC1α*) in interscapular WAT (iWAT) of AA-treated DIO mice (Supplementary Fig. 4b,c,d). However, these markers and *CIDEA* in gonadal WAT (gWAT) were significantly up-regulated by AA (Supplementary Fig. 4b,c,d). Notably, expressions of *CPT1b* and *COX8b* were consistently enhanced in both gWAT and iWAT (Supplementary Fig. 4c,d), suggesting promoted β-oxidation of fatty acids by AA. Importantly, morphological imaging of the adipose tissues (Supplementary Fig. 4b) showed that AA treatment enlarged size of adipocytes in iBAT, iWAT and gWAT along with marked elevation of *PPARγ* expression (Supplementary Fig. 4c).

We next analyzed the metabolic profiles using indirect calorimetry

measurements at the end of AA treatment. During the three-day period of measurements, AA-treated DIO mice exhibited significantly lower levels of oxygen consumption and carbon dioxide production in both dark and light cycles compared to HFD fed mice (Supplementary Fig. 5a,b). Nonetheless, respiratory exchange ratio (RER) revealed a pronounced decrease in mice supplemented with AA (Supplementary Fig. 5c). This result indicated that AA-treated mice consumed more fat as an energy source, which was in line with up-regulated expressions of *COX8b* and *CPT1b* in WAT (Supplementary Fig. 5c,d). Furthermore, these changes occurred with decreased water intake, movement and speed in AA-fed mice (Supplementary Fig. 5d,e,f).

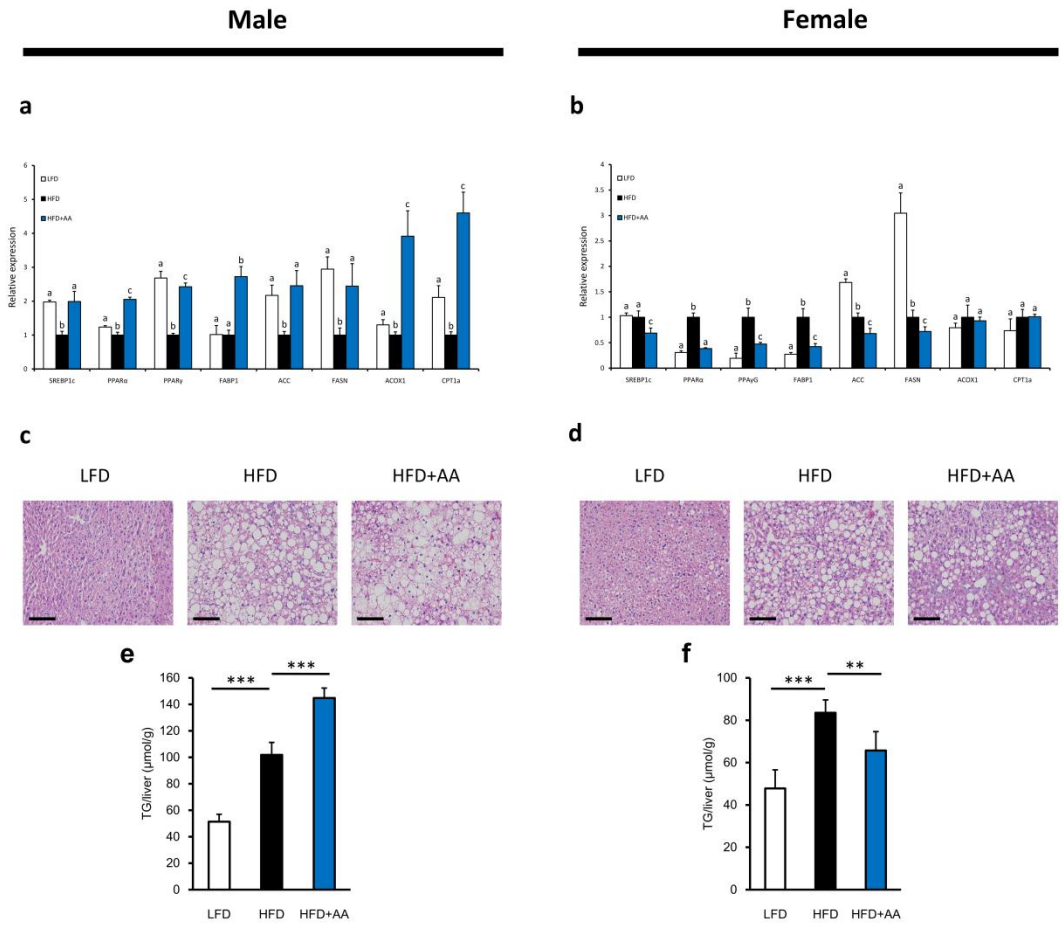


Fig. 5. AA sex-dependently modifies lipid homeostasis (*n*=6). (**a**,**b**) mRNA expression of genes participating hepatic TG metabolism. (**c**,**d**) Representative photomicrographs of liver sections using H-E staining. Scale bar, 100 μm. (**e**,**f**)

Hepatic TG levels. Graph bars with different superscript letters appear significantly different (P<0.05). **P<0.01, ***P<0.001.

3.5. AA impacts obesity-induced non-alcoholic steatohepatitis (NASH) in a sex-specific manner

To investigate the possible obesity-induced NASH, we then assessed whether AA impacts hepatic lipid metabolism. Interestingly, AA markedly exacerbated hepatic TG deposition in male mice (P<0.001) but conversely attenuated TG contents significantly in female mice (P<0.01) (Fig. 5e,f). In addition, circulating TG level was increased by AA in male mice but was not significantly affected in female (Supplementary Fig. 6a,b). Moreover, circulating cholesterol levels were elevated for both genders treated with AA (Supplementary Fig. 6c,d). Nonetheless, supplementation with AA significantly reduced hepatic cholesterol contents in all DIO mice, including TC, HDL-C, LDL-C and VLDL-C (Supplementary Fig. 6e,f). Subsequently, we assessed hepatic lipogenic and oxidative gene expression and documented that the DIO male mice served with AA significantly showed upregulated expression of *ACC* and *FASN*, key genes associated with lipogenesis, along with increased mRNA levels of *SREBP-1c*, *PPARγ* and *PPARα*, and enhanced expression of β-oxidation related genes *ACOX1* and *CPT1α* (Fig. 5a), which may result from a regulatory feedback mechanism. Reversely, we observed decreased expression of *ACC*, *FASN*, *SREBP-1c*, *PPARγ* and *PPARα* though the expression of *ACOX1* and *CPT1α* was not altered in female (Fig. 5b). Notably, hepatic expression of fatty acid-binding protein 1 (*FABP1*) was elevated in AA-treated DIO male mice but reduced in female mice (Fig. 5b). Furthermore, we investigated hepatic levels of miR-122 and miR-33, which regulate hepatic lipid and cholesterol homeostasis, and documented that AA suppressed levels of miR-122 and miR-33 in liver of DIO female mice while no apparent alteration was found in male (Supplementary Fig. 7a,b). Finally, we observed that the liver sections of DIO mice had extensive vacuolization, which was further augmented by AA in male but attenuated in female (Fig. 5c,d).

We then investigated whether AA affected the HFD-induced liver inflammation. Hepatic mRNA levels of *TNF-α*, *IL6*, *CCL2* and *TLR4* were significantly increased in AA-treated male mice but reduced in female mice (Fig. 6a,b). Additionally, IκB-α degradation and phosphorylation of NF-κB and JNK were enhanced in HFD+AA fed male mice whereas inhibited in female mice (Fig. 6g,h).

Overall, these findings suggest that AA exacerbate NASH along with amplified inflammation via TLR4-NF-κB pathway in DIO male mice, but attenuate in female and thus lead to reduced hepatic inflammation and ameliorated NASH.

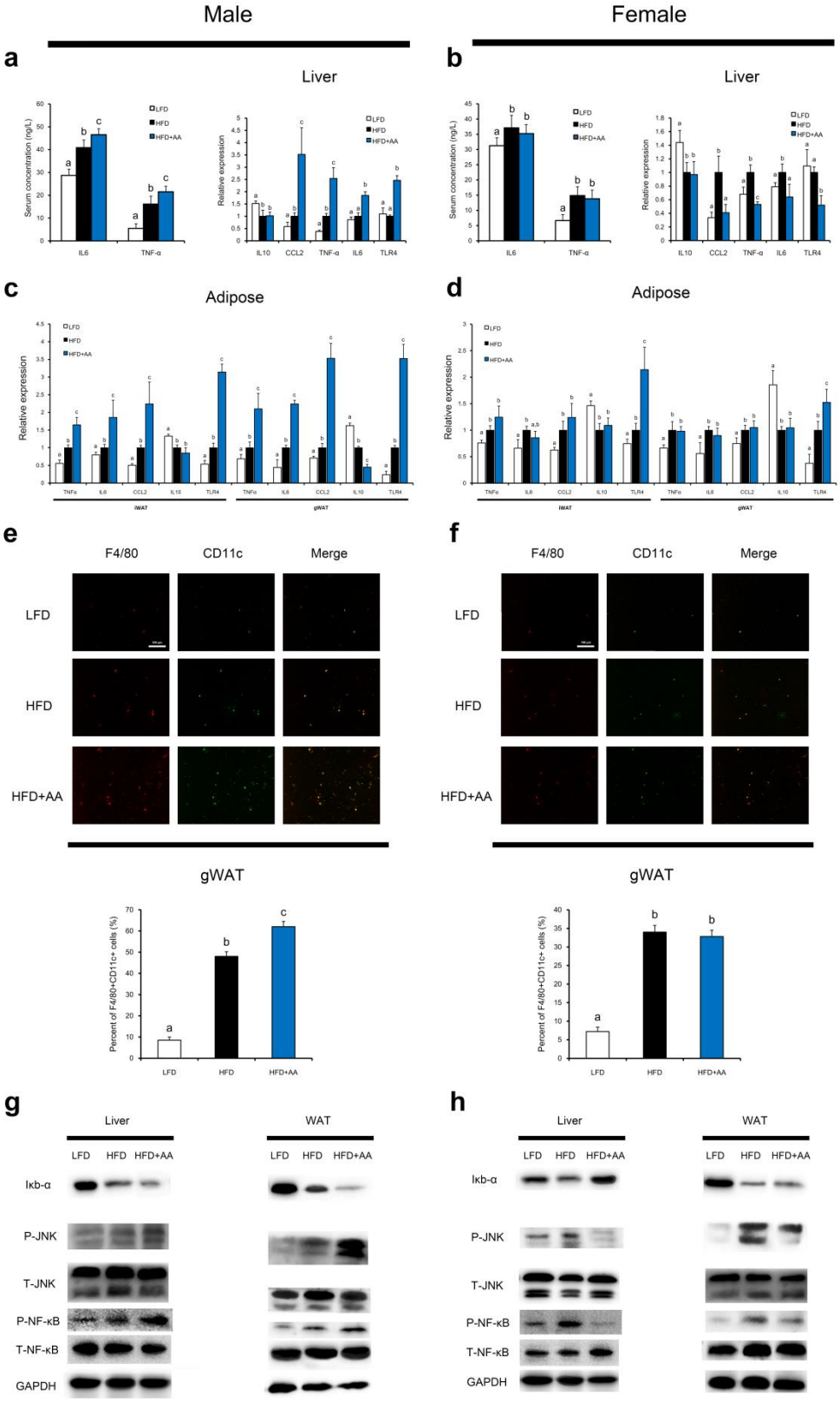


Fig. 6. AA sex-dependently impacts inflammation (*n*=6). (**a**,**b**) Serum concentrations of IL6 and TNFα (left) and hepatic mRNA expression (right). (**c**,**d**) Adipose mRNA

expression. (**e**,**f**) Representative images of gWAT stained with F4/80 and CD11c and the percentage of positive cells. (**g**,**h**) Representative liver and adipose immunoblots. The corresponding densitometry measurements were shown in Supplemental Fig. 1. Graph bars with different superscript letters appear significantly different (P<0.05).

3.6. AA affects glucose homeostasis in DIO mice

We next investigated whether AA also affected the glucose homeostasis. The AA-fed male mice showed higher glucose levels while female mice exhibited lower insulin levels significantly when comparing with those in DIO mice (Fig. 7a,b). Moreover, we observed an ascending trend of hemoglobin A1c levels in HFD+AA fed male mice (Fig. 7a). We also performed both OGTT and ITT and documented that AA-fed male mice were even less glucose tolerant and more insulin resistant than the HFD group while AA-fed female mice exhibited improved IR (Fig. 7c,d,e,f). To further investigate the molecular mechanism, we then assessed the protein expression of glucose transporter 4 (*GLUT4*) in iWAT and gWAT critical for glucose metabolism. As anticipated, *GLUT4* expression was suppressed in male and enhanced in female with AA treatment (Fig. 7g,h). Together, these data indicate that AA aggravates IR for male but oppositely rescues insulin sensitivity for female in DIO mice.

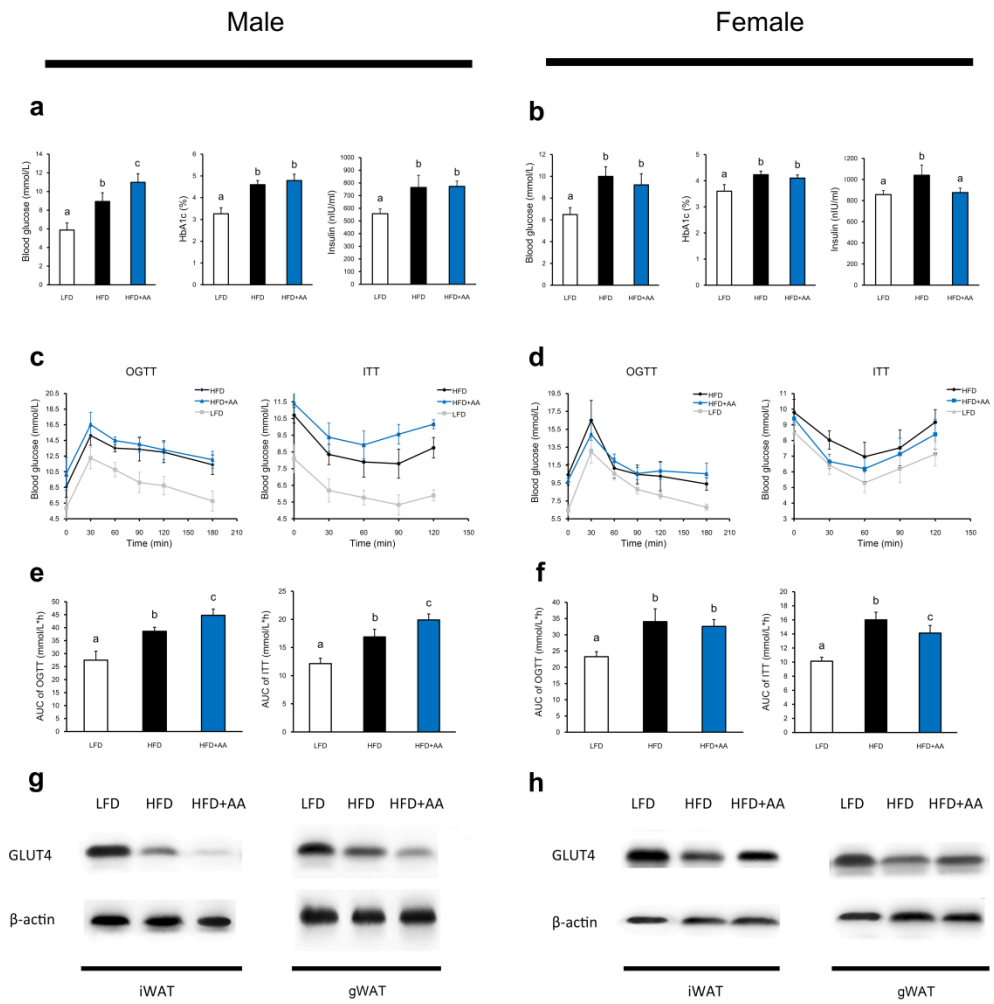


Fig. 7. AA sex-dependently modifies glucose homeostasis (*n*=6). (**a**,**b**) Serum glucose, blood HbA1c and serum insulin levels. (**c**,**d**) Time-dependent profiles of serum glucose levels in OGTT and ITT. (**e**,**f**) The mean AUC from OGTT and ITT. (**g**,**h**) Representative adipose immunoblots for *GLUT4*. The corresponding densitometry measurements were shown in Supplemental Fig. 1. Graph bars with different superscript letters appear significantly different (P<0.05).

Obesity-related inflammation resulting from adipose macrophage infiltration plays a critical role in the pathogenesis of IR [19]. Therefore, we measured proinflammatory cytokines and showed increased circulating levels of *TNF-α* and *IL6* in AA-treated male mice while no apparent alterations were detected in female (Fig. 6a,b). Similarly, we observed increased mRNA levels of *TNF-α*, *IL6* and *CCL2* and decreased expression of *IL10* in both iWAT and gWAT in AA-treated male but not female mice (Fig. 6c,d). We further assessed macrophages infiltration in gWAT and iWAT through $F4/80^+$ and $CD11c^+$ staining. In consistent with the shifts of inflammatory cytokines, F4/80- and CD11c-positive cells were increased in HFD+AA fed male but not female mice (Fig. 6e,f, Supplementary Fig. 8a,b).

Obese adipose tissue is characterized by hypoxic conditions in adipocytes and linked to the progression of IR [20]. Thus we analyzed hypoxia-related gene expression of *HIF1α* and observed an increased protein level of *HIF1α* in both gWAT and iWAT in HFD+AA male but not female mice (Supplementary Fig. 8c). Finally, we also observed promoted expressions of TLR4 (mRNA), phosphorylated NF-κB and JNK, and suppressed IκB-α production in AA-treated DIO male mice but not female mice (Fig. 6g,h).

4. Discussion

Here we demonstrated that long-term intake of AA (1% w/w, corresponding to 417 mg/day for human) aggravates obesity and affects gut microbiota composition, multi-organ inflammation, hepatic lipid metabolism and glucose homeostasis in a sex-dependent way (Supplementary Fig. 9). To our knowledge, we are the first to investigate the gut microbiota alteration by AA and discover the sexual dimorphisms especially in glucose and hepatic lipid homeostasis.

Diet interacts with gut microbiota to modulate human metabolism [21]. In current model of milk fat based DIO mice, the ratio of Firmicutes/Bacteroidetes declined. Importantly, we observed the dysbiosis of intestinal microbiota and increased serum LPS levels, which triggers a global inflammatory internal

environment [11]. Our results showed that AA further reduced the ratio of Firmicutes/Bacteroidetes and impaired intestinal barrier, leading to aggravated metabolic endotoxemia.

AA administration altered series of obesity-associated bacterial species from *Allobaculum* [22], *Oscillibacter* [23], *Oscillospira* [24], *Clostridium* IV and *Bacteroides* [25]. Additionally, we believe that intestinal microbial shifts can be the driver of inflammation. The alterations of inflammation-correlated microbiota, including the increase in *Mucispirillum schaedleri* [26], *Akkermansia muciniphila* [22, 27, 28], *Alistipes* [29] and *Ruminococcus gnavus* [30, 31], and the decrease in *Oscillibacter* [32], *Barnesiella* [33], *Lactobacillus*, *Bifidobacterium* [34] and *Clostridium* XlVa and IV [35], may partially account for the pro-inflammatory effect of AA on HFD-fed male mice (Fig. 2d). Likewise, enhancement in *Barnesiella* and reductions in *Alistipes*, *Ruminococcus gnavus*, *Pseudomonas veronii* [36] and *Akkermansia muciniphila* may partially elucidate the alleviation of inflammation in female.

A previous study showed that fucosyllactose supplementation enriched *Barnesiella* genus in mice and consequently increased the resistance of colitis [33]. Instead, bacteria belonging to *Alistipes* were found to induce colitis and tumorigenesis when transferred into *IL10*$^{-/-}$ mice [29]. In addition, *Ruminococcus gnavus*, an unusual pathogen, was reported to be implicated in cases of diverticular disease [37]. In our study, AA reversed the presence of *Barnesiella*, *Alistipes* and *Ruminococcus gnavus* initially changed by HFD in female but not male mice, indicating that the sex-specific changes of inflammation-correlated microbiota may elucidate the sexual dimorphisms in inflammation.

Butyrate, a SCFA, exerts beneficial effects on various metabolic disorders. β-Hydroxybutyric acid inhibited microglial activation via *GPR109A* and NF-κB signaling in Parkinson's disease models [38]. Butyrate supplementation also attenuated hepatic inflammation and ameliorated NAFLD in rodents [39]. Moreover, butyrate alleviated inflammation by interacting with adipocytes and macrophages in 3T3-L1 adipocytes via *GPR41* [40] and improved IR in mice [41]. Here we observed

the sex-dependent production of butyrate by AA along with no augmented inflammation in hypothalamus and adipose, alleviated hepatic inflammation and steatosis, and improved IR in female mice. Oppositely, butyrate level was reduced in male mice with exacerbated obesity-induced complications. Therefore, it is likely that butyrate plays a critical role in protecting female mice against inflammation aggravation and related metabolic disorders via GPRs binding with butyrate. These also, in turn, highlight that original changes of caecal microbiota may be the key reason accounting for the metabolic alterations. Recently, butyrate has also been shown to increase energy expenditure and ameliorate obesity in mice [42, 43]. Unfortunatey, butyrate could not resist the AA-induced lipogenesis and impaired adipose tissue browning in female mice in our study. We also demonstrated that supplementation with AA reduced and elevated circulating serotonin, in male and female mice, respectively. Peripheral serotonin released by enterochromaffin cells serves as a signaling molecule to modulate biological functions. HFD-fed mice intraperitoneally injected with serotonin relieved obesity and IR by shifting muscle type from glycolytic to oxidative type [44].

Here we showed that AA exacerbated hypothalamic inflammation and decreased leptin sensitivity, which resulted in hyperphagia in DIO male mice. AA further augmented the accumulation of microglia along with elevated expression of pro-inflammatory cytokines and activated NF-κB signaling, suggesting that microglia may also mediate the impact of AA on leptin sensitivity and food intake [45]. In addition, we observed a decreased circulating leptin level accompanying with increased adipose tissue mass in both genders of obese mice. A previous study demonstrated the inhibitory effect of AA on leptin secretion and expression in isolated adipocytes, which might be related to the alterations in the metabolic utilization of glucose [46]. Here we also show a significant inhibitory effect of AA on leptin, which may be an important contributor to aggravated obesity.

Importantly, AA administration is able to prevent HFD induced IR by maintaining membrane integrity and normal intercellular signaling [47]. AA could also stimulate

glucose uptake by increasing the expression of *GLUT1* and *GLUT4* in 3T3-L1 adipocytes [48]. Here we found that AA decreased insulin sensitivity in male mice due to aggravated inflammation and its interplay with reduced *GLUT4* content. Also, hypoxia and activated NF-κB signaling induced by AA contributed to WAT microphages infiltration and thus augmented adipose inflammation in male mice. Interestingly, the selected dosage of AA here was efficient to promote *GLUT4* expression in adipose without inducing adipose inflammation in female. This indicates a gender- and dose-dependent manner of AA in regulating glucose homeostasis.

NAFLD is also an obesity-induced health problem ranking from simple steatosis to NASH differentiated by inflammatory cell infiltration. In our model, AA-fed male mice showed similar increases in serum TC, TG and LDL-C as well as aggravated hepatic expansion and steatohepatitis. Oppositely, AA ameliorated steatohepatitis with attenuated hepatic inflammation in female. Thus, we propose that AA-modulated inflammation be the key reason for elucidating gender-dependent performance of liver pathology in DIO mice. Growing evidence has revealed that miR-122 and miR-33 play a predominant role in liver disorders. Therapeutic silencing of miR-33 in mice promotes fatty acid β-oxidation [49]. Similarly, miR-122 participates in the accumulation of hepatic triglycerides by promoting lipogenesis and inhibiting fatty acid oxidation [50]. Here we proved that AA reduced hepatic expression of miR-122 and miR-33 in female mice. Moreover, their targeted gene *SREBP-1c* and downstream genes *FAS* and *ACC* were also down-regulated. Therefore, AA-mediated miR-122 and miR-33 suppression is likely another mechanism of action of improving NAFLD in female mice.

In view of multi-organ response to AA supplementation, the gut interplays with brain, adipose and liver in DIO mice. We suggest that AA modulate gut microbiota to affect the production of cytokines (e.g. *TNFα* and *IL6*) in intestinal immune cells, the release of LPS, and the generation of SCFA and serotonin. These signaling molecules impact the inflammation response, in hypothalamus, adipose and liver, and regulate

lipid and glucose homeostasis. Besides, anti-adipogenic molecules including insulin, adipokine leptin and adiponectin exert anorexigenic effect in hypothalamus while orexigenic brain peptide *AGRP* and *NPY* favor lipogenesis in adipose tissue [51]. Moreover, the inflammatory etiology of hypothalamus and adipose contributes to pathogenesis of NAFLD [52]. Collectively, the role of AA in DIO mice involves complicated interactions among gut, brain, adipose and liver.

It has been documented that the diet-sex interaction controls host microbiota composition possibly due to sex hormones and sex differences in immune function [53]. At first glance, sex hormones could modulate microbiota composition and thus generate systematic gut microbial differences between male and female mice in our study. Furthermore, the sex-diet interaction appears possible if sex hormone-dependent microbes are also affected by host diet [53]. In the present case, the sex hormone-dependent microbes may also be affected by AA, resulting in a sex-AA interaction. In addition, we propose that AA sex-dependently modulates microbiota composition which is in favor or disfavor of inflammation/obesity-correlated species. This two-directional outcome led to sexual dimorphisms in inflammation, which manifests as the underlined mechanism explaining the sexually dimorphic phenotype of metabolic disorders. In addition, subsequent alterations of SCFA, serotonin and cytokines (e.g. *TNFα* and *IL6*) production as well as microRNA expression are also involved.

5. Conclusion

In conclusion, our results show that long-term supplementation with AA aggravates existing obesity in HFD-fed mice. Notably, obese male mice are subject to the adverse effect of AA on obesity-induced complications whereas female NAFLD and IR are improved. AA may mediate these sex-dependent effects via gut-brain/adipose/liver axis. Importantly, the alteration of gut microbiota-driven inflammation may at least partially contribute to AA-induced metabolic regulations. Collectively, these results highlight the need for further clinical studies to preferably

formulate recommendations of n-6 PUFA intakes for obese male and female separately.

Conflicts of interest statement

The authors declare no conflict of interest.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (31401659, 31201307 and 81300309). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References

[1] A. Astrup, N. Finer, Redefining Type 2 diabetes: 'Diabesity' or 'Obesity Dependent Diabetes Mellitus'?, Obes. Rev. 1 (2000) 57-59.

[2] J.H. Ix, K. Sharma, Mechanisms linking obesity, chronic kidney disease, and fatty liver disease: the roles of fetuin-A, adiponectin, and AMPK, J. Am. Soc. Nephrol. 21 (2010) 406-412.

[3] A.G. Bertoni, G.L. Burke, J.A. Owusu, M.R. Carnethon, D. Vaidya, R.G. Barr, N.S. Jenny, P. Ouyang, J.I. Rotter, Inflammation and the incidence of type 2 diabetes: the Multi-Ethnic Study of Atherosclerosis (MESA), Diabetes Care 33 (2010) 804-810.

[4] Marc Y. Donath, É. Dalmas, Nadine S. Sauter, M. Böni-Schnetzler, Inflammation in obesity and diabetes: islet dysfunction and therapeutic opportunity, Cell Metab. 17 (2013) 860-872.

[5] J.P. Thaler, C.-X. Yi, E.A. Schur, S.J. Guyenet, B.H. Hwang, M.O. Dietrich, X. Zhao, D.A. Sarruf, V. Izgur, K.R. Maravilla, H.T. Nguyen, J.D. Fischer, M.E. Matsen, B.E. Wisse, G.J. Morton, T.L. Horvath, D.G. Baskin, M.H. Tschöp, M.W. Schwartz, Obesity is associated with hypothalamic injury in rodents and humans, J. Clin. Invest. 122 (2012) 153-162.

[6] L. Gautron, J.K. Elmquist, K.W. Williams, Neural control of energy balance: translating circuits to therapies, Cell 161 (2015) 133-145.

[7] S. Musatov, W. Chen, D.W. Pfaff, C.V. Mobbs, X.-J. Yang, D.J. Clegg, M.G. Kaplitt, S. Ogawa, Silencing of estrogen receptor α in the ventromedial

nucleus of hypothalamus leads to metabolic syndrome, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 2501-2506.

[8] Y. Xu, T.P. Nedungadi, L. Zhu, N. Sobhani, B.G. Irani, K.E. Davis, X. Zhang, F. Zou, L.M. Gent, L.D. Hahner, S.A. Khan, C.F. Elias, J.K. Elmquist, D.J. Clegg, Distinct hypothalamic neurons mediate estrogenic effects on energy homeostasis and reproduction, Cell Metab. 14 (2011) 453-465.

[9] E. Morselli, E. Fuente-Martin, B. Finan, M. Kim, A. Frank, C. Garcia-Caceres, C.R. Navas, R. Gordillo, M. Neinast, S.P. Kalainayakan, Y. Gao, C.-X. Yi, L. Hahner, B.F. Palmer, M.H. Tschöp, D.J. Clegg, Hypothalamic PGC-1α protects against high fat diet exposure by regulating ERα, Cell Rep. 9 (2014) 633-645.

[10] V.K. Ridaura, J.J. Faith, F.E. Rey, J. Cheng, A.E. Duncan, A.L. Kau, N.W. Griffin, V. Lombard, B. Henrissat, J.R. Bain, M.J. Muehlbauer, O. Ilkayeva, C.F. Semenkovich, K. Funai, D.K. Hayashi, B.J. Lyle, M.C. Martini, L.K. Ursell, J.C. Clemente, W. Van Treuren, W.A. Walters, R. Knight, C.B. Newgard, A.C. Heath, J.I. Gordon, Cultured gut microbiota from twins discordant for obesity modulate adiposity and metabolic phenotypes in mice, Science 341 (2013) 1241214.

[11] P.D. Cani, R. Bibiloni, C. Knauf, A. Waget, A.M. Neyrinck, N.M. Delzenne, R. Burcelin, Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice, Diabetes 57 (2008) 1470-1481.

[12] H. Shi, M.V. Kokoeva, K. Inouye, I. Tzameli, H. Yin, J.S. Flier, TLR4 links innate immunity and fatty acid-induced insulin resistance, J. Clin. Invest. 116 (2006) 3015-3025.

[13] B.T. Layden, A.R. Angueira, M. Brodsky, V. Durai, W.L. Lowe Jr, Short chain fatty acids and their receptors: new metabolic targets, Transl. Res. 161 (2013) 131-140.

[14] V. Ridaura, Y. Belkaid, Gut microbiota: the link to your second brain, Cell 161 (2015) 193-194.

[15] F. De Vadder, P. Kovatcheva-Datchary, D. Goncalves, J. Vinera, C. Zitoun, A. Duchampt, F. Bäckhed, G. Mithieux, Microbiota-generated metabolites promote metabolic benefits via gut-brain neural circuits, Cell 156 (2014) 84-96.

[16] P.Y.T. Wang, L. Caspi, C.K.L. Lam, M. Chari, X. Li, P.E. Light, R. Gutierrez-Juarez, M. Ang, G.J. Schwartz, T.K.T. Lam, Upper intestinal lipids trigger a gut-brain-liver axis to regulate glucose production, Nature 452 (2008) 1012-1016.

[17] W.S. Powell, J. Rokach, Biosynthesis, biological effects, and receptors of hydroxyeicosatetraenoic acids (HETEs) and oxoeicosatetraenoic acids (oxo-ETEs) derived from arachidonic acid, Biochim. Biophys. Acta-Mol. Cell Biol. Lipids 1851 (2015) 340-355.

[18] C. Chevalier, O. Stojanović, D.J. Colin, N. Suarez-Zamorano, V. Tarallo, C. Veyrat-Durebex, D. Rigo, S. Fabbiano, A. Stevanović, S. Hagemann, X.

Montet, Y. Seimbille, N. Zamboni, S. Hapfelmeier, M. Trajkovski, Gut microbiota orchestrates energy homeostasis during cold, Cell 163 (2015) 1360-1374.

[19] C.N. Lumeng, J.L. Bodzin, A.R. Saltiel, Obesity induces a phenotypic switch in adipose tissue macrophage polarization, J. Clin. Invest. 117 (2007) 175-184.

[20] N. Hosogai, A. Fukuhara, K. Oshima, Y. Miyata, S. Tanaka, K. Segawa, S. Furukawa, Y. Tochino, R. Komuro, M. Matsuda, I. Shimomura, Adipose tissue hypoxia in obesity and its impact on adipocytokine dysregulation, Diabetes 56 (2007) 901-911.

[21] J.L. Sonnenburg, F. Backhed, Diet-microbiota interactions as moderators of human metabolism, Nature 535 (2016) 56-64.

[22] Y.Y. Lam, C.W.Y. Ha, J.M.A. Hoffmann, J. Oscarsson, A. Dinudom, T.J. Mather, D.I. Cook, N.H. Hunt, I.D. Caterson, A.J. Holmes, L.H. Storlien, Effects of dietary fat profile on gut permeability and microbiota and their relationships with metabolic changes in mice, Obesity 23 (2015) 1429-1439.

[23] B.-S. Kim, M.-Y. Song, H. Kim, The anti-obesity effect of Ephedra sinica through modulation of gut microbiota in obese Korean women, J. Ethnopharmacol. 152 (2014) 532-539.

[24] T. Konikoff, U. Gophna, Oscillospira: a central, enigmatic component of the human gut microbiota, Trends Microbiol. 24 (2016) 523-524.

[25] C.-J. Chang, C.-S. Lin, C.-C. Lu, J. Martel, Y.-F. Ko, D.M. Ojcius, S.-F. Tseng, T.-R. Wu, Y.-Y.M. Chen, J.D. Young, H.-C. Lai, Ganoderma lucidum reduces obesity in mice by modulating the composition of the gut microbiota, Nat. Commun. 6 (2015) 7489.

[26] A. Everard, V. Lazarevic, N. Gaïa, M. Johansson, M. Ståhlman, F. Backhed, N.M. Delzenne, J. Schrenzel, P. François, P.D. Cani, Microbiome of prebiotic-treated mice reveals novel targets involved in host response during obesity, ISME J. 8 (2014) 2116-2130.

[27] D. Berry, O. Kuzyk, I. Rauch, S. Heider, C. Schwab, E. Hainzl, T. Decker, M. Müller, B. Strobl, C. Schleper, T. Urich, M. Wagner, L. Kenner, A. Loy, Intestinal microbiota signatures associated with inflammation history in mice experiencing recurring colitis, Front. Microbiol. 6 (2015) 1408.

[28] B.P. Ganesh, R. Klopfleisch, G. Loh, M. Blaut, Commensal Akkermansia muciniphila exacerbates gut inflammation in salmonella typhimurium-infected gnotobiotic mice, PLoS One 8 (2013) e74963.

[29] Alexander R. Moschen, Romana R. Gerner, J. Wang, V. Klepsch, Timon E. Adolph, Simon J. Reider, H. Hackl, A. Pfister, J. Schilling, Patrizia L. Moser, Sarah L. Kempster, A. Swidsinski, D. Orth-Höller, G. Weiss, John F. Baines, A. Kaser, H. Tilg, Lipocalin 2 protects from inflammation and tumorigenesis associated with gut microbiota alterations, Cell Host Microbe 19 (2016) 455-469.

[30] B.A. Petriz, A.P. Castro, J.A. Almeida, C.P.C. Gomes, G.R. Fernandes, R.H.

Kruger, R.W. Pereira, O.L. Franco, Exercise induction of gut microbiota modifications in obese, non-obese and hypertensive rats, BMC Genomics 15 (2014) 511.

[31] M. Titécat, F. Wallet, M.-H. Vieillard, R.J. Courcol, C. Loïez, Ruminococcus gnavus: an unusual pathogen in septic arthritis, Anaerobe 30 (2014) 159-160.

[32] J. Li, C.Y.J. Sung, N. Lee, Y. Ni, J. Pihlajamäki, G. Panagiotou, H. El-Nezami, Probiotics modulated gut microbiota suppresses hepatocellular carcinoma growth in mice, Proc. Natl. Acad. Sci. U. S. A. 113 (2016) E1306-E1315.

[33] G.A. Weiss, C. Chassard, T. Hennet, Selective proliferation of intestinal Barnesiella under fucosyllactose supplementation in mice, Br. J. Nutr. 111 (2014) 1602-1610.

[34] S. Giardina, C. Scilironi, A. Michelotti, A. Samuele, F. Borella, M. Daglia, F. Marzatico, In vitro anti-inflammatory activity of selected oxalate-degrading probiotic bacteria: potential applications in the prevention and treatment of hyperoxaluria, J. Food Sci. 79 (2014) M384-M390.

[35] K. Atarashi, T. Tanoue, K. Oshima, W. Suda, Y. Nagano, H. Nishikawa, S. Fukuda, T. Saito, S. Narushima, K. Hase, S. Kim, J.V. Fritz, P. Wilmes, S. Ueha, K. Matsushima, H. Ohno, B. Olle, S. Sakaguchi, T. Taniguchi, H. Morita, M. Hattori, K. Honda, Treg induction by a rationally selected

mixture of Clostridia strains from the human microbiota, Nature 500 (2013) 232-236.

[36] W. Cheuk, P.C.Y. Woo, K.Y. Yuen, P.H. Yu, J.K.C. Chan, Intestinal inflammatory pseudotumour with regional lymph node involvement: identification of a new bacterium as the aetiological agent, J. Pathol. 192 (2000) 289-292.

[37] S.G.K. Hansen, M.N. Skov, U.S. Justesen, Two cases of Ruminococcus gnavus bacteremia associated with diverticulitis, J. Clin. Microbiol. 51 (2013) 1334-1336.

[38] S.-P. Fu, J.-F. Wang, W.-J. Xue, H.-M. Liu, B.-r. Liu, Y.-L. Zeng, S.-N. Li, B.-X. Huang, Q.-K. Lv, W. Wang, J.-X. Liu, Anti-inflammatory effects of BHBA in both in vivo and in vitro Parkinson's disease models are mediated by GPR109A-dependent mechanisms, J. Neuroinflammation 12 (2015) 9.

[39] C.J. Jin, C. Sellmann, A.J. Engstler, D. Ziegenhardt, I. Bergheim, Supplementation of sodium butyrate protects mice from the development of non-alcoholic steatohepatitis (NASH), Br. J. Nutr. 114 (2015) 1745-1755.

[40] Y.F. Hideo Ohira, Chikae Katagiri, Rie Mamoto, Michiko Aoyama-Ishikawa, Katsumi Amako, Yoshihiro Izumi, Shin Nishiumi, Masaru Yoshida, Makoto Usami, Masamichi Ikeda, Butyrate attenuates inflammation and lipolysis generated by the interaction of adipocytes and macrophages, J. Atheroscler. Thromb. 20 (2013) 425-442.

[41] X. Wang, G. He, Y. Peng, W. Zhong, Y. Wang, B. Zhang, Sodium butyrate alleviates adipocyte inflammation by inhibiting NLRP3 pathway, Sci. Rep. 5 (2015) 12676.

[42] Z. Gao, J. Yin, J. Zhang, R.E. Ward, R.J. Martin, M. Lefevre, W.T. Cefalu, J. Ye, Butyrate improves insulin sensitivity and increases energy expenditure in mice, Diabetes 58 (2009) 1509-1517.

[43] M.P. Mollica, G.M. Raso, G. Cavaliere, G. Trinchese, C. De Filippo, S. Aceto, M. Prisco, C. Pirozzi, F. Di Guida, A. Lama, M. Crispino, D. Tronino, P. Di Vaio, R.B. Canani, A. Calignano, R. Meli, Butyrate regulates liver mitochondrial function, efficiency, and dynamics in insulin-resistant obese mice, Diabetes 66 (2017) 1405-1418.

[44] H. Watanabe, T. Nakano, R. Saito, D. Akasaka, K. Saito, H. Ogasawara, T. Minashima, K. Miyazawa, T. Kanaya, I. Takakura, N. Inoue, I. Ikeda, X. Chen, M. Miyake, H. Kitazawa, H. Shirakawa, K. Sato, K. Tahara, Y. Nagasawa, M.T. Rose, S. Ohwada, K. Watanabe, H. Aso, Serotonin improves high fat diet induced obesity in mice, PLoS One 11 (2016) e0147143.

[45] M. Valdearcos, M.M. Robblee, D.I. Benjamin, D.K. Nomura, A.W. Xu, S.K. Koliwad, Microglia dictate the impact of saturated fat consumption on hypothalamic inflammation and neuronal function, Cell Rep. 9 (2014) 2124-2138.

[46] P. Pérez-Matute, A. Marti, J.A. Martínez, M.J. Moreno-Aliaga, Effects of

arachidonic acid on leptin secretion and expression in primary cultured rat adipocytes, J. Physiol. Biochem. 59 (2003) 201-208.

[47] M. Wu, X. Wang, Q. Duan, T. Lu, Arachidonic acid can significantly prevent early insulin resistance induced by a high-fat diet, Ann. Nutr. Metab. 51 (2007) 270-276.

[48] C. Nugent, J.B. Prins, J.P. Whitehead, J.M. Wentworth, V.K.K. Chatterjee, S. O'Rahilly, Arachidonic acid stimulates glucose uptake in 3T3-L1 adipocytes by increasing GLUT1 and GLUT4 levels at the plasma membrane: evidence for involvement of lipoxygenase metabolites and peroxisome proliferator-activated receptor γ, J. Biol. Chem. 276 (2001) 9149-9157.

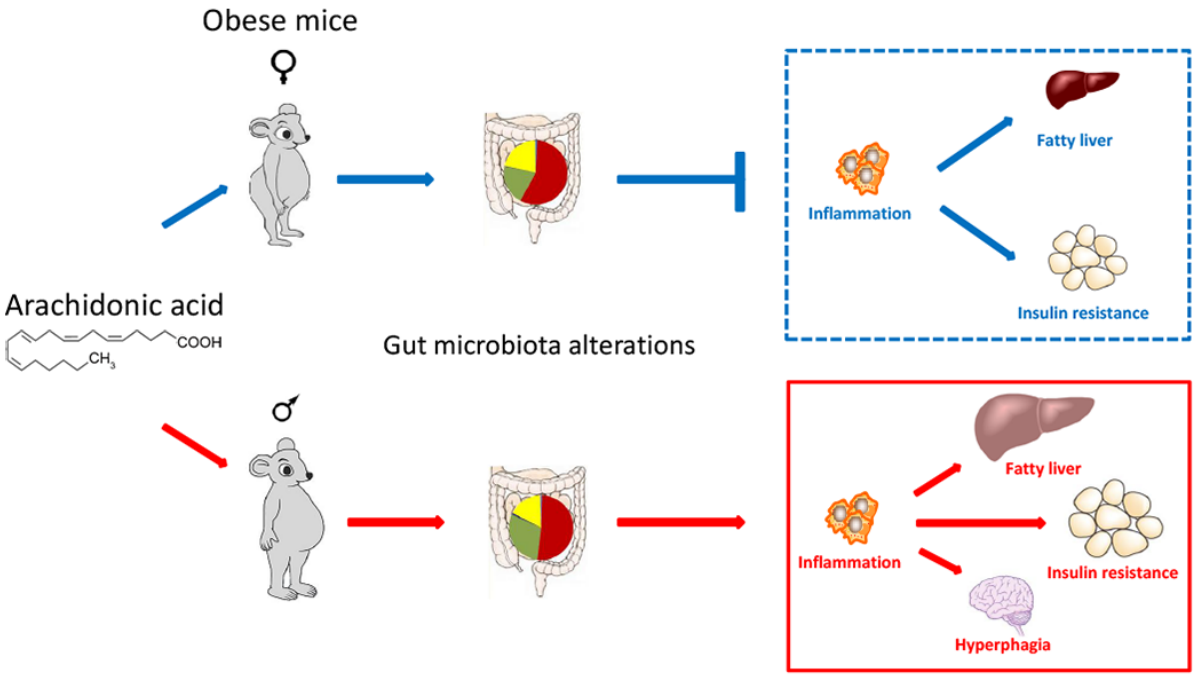
[49] D. Karunakaran, L. Richards, M. Geoffrion, D. Barrette, R.J. Gotfrit, M.-E. Harper, K.J. Rayner, Therapeutic inhibition of miR-33 promotes fatty acid oxidation but does not ameliorate metabolic dysfunction in diet-induced obesity, Arterioscler. Thromb. Vasc. Biol. 35 (2015) 2536-2543.

[50] D. Iliopoulos, K. Drosatos, Y. Hiyama, I.J. Goldberg, V.I. Zannis, MicroRNA-370 controls the expression of MicroRNA-122 and Cpt1α and affects lipid metabolism, J. Lipid Res. 51 (2010) 1513-1523.

[51] H. Shimizu, M. Mori, The brain-adipose axis: a review of involvement of molecules, Nutr. Neurosci. 8 (2005) 7-20.

[52] P.I. Mighiu, B.M. Filippi, T.K.T. Lam, Linking inflammation to the brain-liver axis, Diabetes 61 (2012) 1350-1352.

[53] D.I. Bolnick, L.K. Snowberg, P.E. Hirsch, C.L. Lauber, E. Org, B. Parks, A.J. Lusis, R. Knight, J.G. Caporaso, R. Svanbäck, Individual diet has sex-dependent effects on vertebrate gut microbiota, Nat. Commun. 5 (2014) 4500.



Graphical abstract

Highlights

1. AA aggravates existing obesity and modifies adipocyte browning.
2. AA may sex-dependently affect obesity-driven inflammation by altering microbiota.
3. AA exacerbates male inflammation and obesity-induced complications.
4. AA ameliorates female fatty liver and insulin resistance with GPR41 up-regulation.