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Original Research

Modulation of gut microbiota by antibiotics did not affect anhedonia in a high-fat diet-induced model of depression in male mice

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Highlights

- Diet-induced obese (DIO) mice were anhedonic and had an altered gut microbiota.
- Antibiotic treatment in DIO mice profoundly altered their gut microbiota.
- Antibiotic treatment in DIO mice did not modify their anhedonia level.
- Four bacterial families may be associated with anhedonia in DIO mice.

Abstract:

Background: Long-term consumption of a high-fat diet (HFD) causes obesity and is a risk factor for depression. The HFD has a significant impact on the gut microbiota, and dysbiosis of the microbiota is now associated with certain psychiatric disorders such as anxiety and depression. We aimed to investigate whether modulation by antibiotic treatment of the composition of the gut microbiota in diet-induced obese (DIO) C57BL/6J male mice has an impact on depressive-like behaviour.

Methods: In this study, we have analysed the effects of a 15 weeks HFD on helplessness assessed in the forced swim test and anhedonia assessed in the sucrose preference test. Two weeks before the start of the behavioural tests, a group of HFD mice were given a combination of two non-absorbable antibiotics, neomycin and polymyxin B.

Results: In DIO mice, anhedonia and significant changes in the composition of the gut microbiota at the phyla and family level were observed. On the other hand, there was no significant effect of HFD on the peripheral inflammatory profile. In DIO mice, antibiotic treatment resulted in very pronounced alteration in the composition of the gut microbiota, without any change in anhedonia behaviour.

Conclusion: In DIO mice, only four families of bacteria were not affected in their relative abundance by the antibiotic treatment, the Bifidobacteriaceae, Erysipelotrichaceae, Rikenellaceae and Streptococcaceae. This stability concomitant with that of anhedonia suggests that these families may be involved in anhedonia in DIO mice.

Keywords: Diet-induced obese mice, Gut microbiota, Antibiotic, Depression, Anhedonia

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Introduction

Obesity and depression are two diseases with a high prevalence and mortality risk. There is increasing evidence of a possible association between these two pathologies, but the causal link has not been established, as no unanimous agreement has yet emerged [1-4]. This association has also been observed in rodent studies with a high-fat diet (HFD), where helplessness and anhedonia were induced in addition to obesity (For review, see [5]). The gut microbiota, whose involvement in obesity and psychiatric disorders has been increasingly investigated, could play a role in this comorbidity [6]. Many studies have shown that the gut microbiota profile was different in obese people compared to lean people. Nowadays, the contribution of the gut microbiota to the development of obesity is well documented by human and animal studies (For review, see [7-8]). In particular, it has been proposed that gram-negative bacteria containing lipopolysaccharide (LPS) present in the gut could play a key role in obesity. Cani et al. [9] showed that subcutaneous infusion of a low dose of LPS, a component of the cell wall of gram-negative bacteria, caused obese and insulin-resistant phenotypes when subcutaneously infused into mice. Through chronic stimulation of intestinal Toll-like receptors 4, The LPS-containing gram-negative bacteria present in the gut could exacerbate the low-grade inflammation associated with obesity and insulin resistance (for review [10]). More recently, several studies have identified differences in the diversity or relative abundances of some bacterial groups in patients suffering from major depressive disorder compared to healthy subjects, which suggests a possible involvement of the gut microbiota in depression (For review, see [11-13]). In pre-clinical studies, depression-like behaviours were described in germ-free rodents receiving a fecal microbiota transplantation derived either from a rodent model of depression [14-15] or from patients suffering from major depressive disorder [16-17].

The aim of our study is to investigate whether the gut microbiota could play a pivotal role in the interaction between obesity and depression. We have designed an experiment to examine whether the modulation of the composition of the gut microbiota by an antibiotic treatment would have an impact on depressive-like traits or behaviours in DIO mice made obese after 11 weeks of HFD consumption (Figure 1). Three groups of mice were established: the STDD-H2O group fed with a standard diet, the HFD-H2O group and HFD-AB group fed with HFD, the latter group received in addition antibiotics. To date, few studies have used antibiotic treatment to investigate the effect of altered gut microbiota on depressive-like behaviour in healthy mice, and with conflicting results [18-20]. To our knowledge, only one study from Hassan et al. [21], has tested the effect of an antibiotic treatment on depressive-like behaviour in DIO mice; the administration of a combination of 3 non-absorbable antibiotics (meropenem 1 mg.ml-1, neomycin 5 mg.ml-1, vancomycin 0.3 mg.ml-1) that extensively depleted gut bacteria, was accompanied by a correction of anhedonia. We chose two non-absorbable antibiotics, neomycin and polymyxin B, which were added to the drinking water of mice in the HFD-AB group two weeks before beginning the behavioural tests. This combination of antibiotics was chosen because it has previously been shown to modify the gut microbiota without reducing drastically the overall bacterial concentration in the gut. In addition, it particularly reduced enterobacteria, which are main LPS producers, and improved obesity associated metabolic disorders in DIO mice [10]. The gut microbiota composition in the 3 groups were ascertained using amplicon sequencing of the 16S rRNA marker gene (metabarcoding). The selected markers of depression-like condition were the coat state, the helplessness (poor coping ability) in the forced swim test, and the anhedonia (inability to experience pleasure in normally pleasurable activities) in the sucrose test. The aim was to assess the effects of the HFD and antibiotic treatment, and to identify families of bacteria associated with depression-like traits in DIO mice.

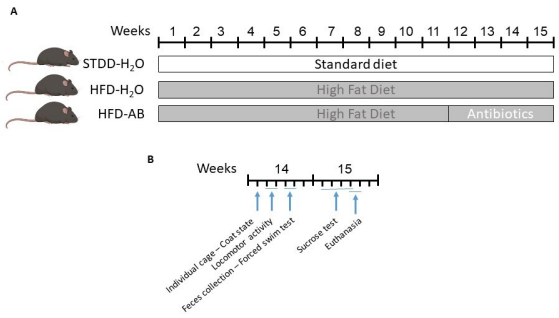


Figure 1. Experimental timeline. (A) General overview. (B) Schedule for the last two weeks of the experiment.

Material and methods

Animals

Forty-five four-week-old C57BL/6J male mice (Janvier Labs, Le Genest Saint Isle, France) were housed, five mice per cage, under standard conditions (12:12h light–dark cycle, lights on at 7:30 AM, 22°C). All animals had free access to the food and water. Procedures were carried out in the Experimental Unit of Rodents and Fish (IERP, INRAE, Jouy-en-Josas, France) in accordance with the European guidelines for the care and use of laboratory animals; they were approved by the local Ethics Committee (Comethea, approval # 14-28).

Animal experimental design

After 1 week of acclimatization, mice were weighed to be allocated to three groups of similar weight. From then, the STDD-H2O group received standard diet 10 kcal% fat, 20 kcal% protein and 70 kcal% carbohydrate (STD Standard Rodent Diet A04, SAFE, Augy, France) ; the HFD-H2O and HFD-AB groups received a HFD with 45 kcal% fat, 20 kcal% protein and 35 kcal% carbohydrate (D12451; Energy

density: 4.7 kcal/g; Research Diets Inc., New Brunswick, NJ, USA) (Figure 1). From the week-12 of the controlled diets, the HFD-AB mice received an antibiotic treatment in their drinking water while the other groups kept regular drinking water. Such non-absorbable antibiotics were reported to be without direct effect on the gut or the central nervous system [22]. The mice and food were weighed every week. At the beginning of week-14 of the controlled diets, the mice were housed in individual cages and had access to two bottles of water to acclimate them to the set-up used to test sucrose preference in week-15. Week-14, Monday, the coat state of mice was evaluated. Week-14, Tuesday or Wednesday, locomotor activity of the mice was measured. Two days later, the helplessness was assessed in the forced swim test. The morning, before this test, fresh feces were collected from each mouse for 16S rRNA gene sequencing. For faecal collections, each mouse is placed alone for a few minutes in a white plastic jar (13 cm high, 10 cm diameter) to obtain 4-5 feces. Week-15, (from Monday or Tuesday) started a 3-day sucrose preference test. Week-15, Thursday or Friday, mice were transferred to a necropsy room for euthanasia.

Antibiotic treatment

From week-12 to the end of the experiment, the non-absorbable antibiotics neomycin sulfate (Fisher Scientific, Illkirch, France) and polymyxin B sulfate (AMS Biotechnology, Abingdon, UK) were added to the drinking water of the HFD-AB mice, at 1 g.l-1 and 0.5 g.l-1, respectively [10]. Fresh solutions were prepared every two days.

Coat state

The coat state of mice was assessed in eight different body parts: head, neck, dorsal coat, ventral coat, tail, forepaws, hindpaws and genital region. A score of 0 for a coat in a good state or a score of 1 for a dirty coat were given for each of these areas. Total score was obtained from the sum of the scores of each body part [23].

Locomotor activity

The test was conducted in a 100 lux illuminated room. Mice were placed in a corner of a rectangular arena with transparent walls (19 cm high, 21 cm large, 37 cm long) whose floor was divided into 5 x 7 squares. The mice were videotaped for 10 min by a camera positioned above the arena. The number of squares crossed, rearings, groomings and defecations were scored manually by an experimenter blinded to experimental groups.

Forced swim test

The procedure consisted of one swimming session, the mice were placed in a transparent glass cylinder (height 24 cm, diameter 12 cm) filled with water (23±1°C) up to 12 cm from the bottom. The mice were videotaped for 6 min by a camera positioned horizontal to the mouse. The duration of immobility, indicator of the helplessness of the mice, was assessed manually by an experimenter blinded to experimental groups. A mouse was judged to be immobile when it ceased struggling and swimming and remained floating motionless in the water making only movements necessary to keep its head above water [24]. At the end of the swimming sessions, the mice were removed from the cylinders, dried with towels, placed in cages for 15 min rest and recovery, and then returned to their home cages. The procedure consisted of one swimming session.

Sucrose preference

Anhedonia was evaluated by measuring sucrose consumption during three consecutive days in mice individually housed in their home cage, using the two-bottle free choice test. The week preceding the test, each mouse had access to two bottles of water. It was then checked that the mice drank equally at the two bottles. Week-15, Monday or Tuesday, water was replaced in one bottle by a 4 % sucrose solution. To prevent possible effects of side preference in drinking behaviour, the positions of the two bottles were swapped each day for 3 days. No food or water deprivation was applied before the test. For the mice of the HFD-AB group, both bottles during habituation and test period contained the antibiotics (neomycin sulfate 1g.l-1; polymyxin B sulfate 0.5 g.l-1). Preference for sucrose was expressed as the ratio (%) of sucrose consumption over total liquid (sucrose plus water) consumption [25].

Euthanasia and blood and brain collection

Mice were euthanized by decapitation. The truncal blood was collected in a plastic tube containing 20 µl EDTA 0.5M, pH 8. Plasma was collected after centrifugation (1000 g, 10 min, 4°C) and stored in aliquots at -80°C. After sacrifice, the caecum was dissected; it was weighed full then empty. The caecal contents were frozen in liquid nitrogen and stored at -80°C.

Measurement of cytokines in plasma

As previously described, plasma TNF-α, IL-1β and IL-6 levels were measured with the Mouse Cytokine/Chemokine magnetic bead panel multiplex assay (Millipore, France) following manufacturer's instructions [26]. All samples were run in duplicate. At the kit detection limit values, the sensitivities of the assay were 2.3, 5.4 and 1.1 pg/mL for TNF-α, IL-1 β and IL-6, respectively.

Fecal metagenomic DNA extraction, 16S rRNA gene amplification and sequencing, and bioinformatic analysis

The bacterial DNA was extracted from faeces of ten mice per group using Genome DNA Isolation Kit (MP Biomedicals, Santa Ana, CA, USA). DNA was resuspended in 100 µl TE (10 mM Tris pH 7.5, 1 mM EDTA) and stored at -20°C. DNA was quantified using the Nanodrop 1000 spectrophotometer (Thermo Scientific). The V3-V4 region of the 16S rRNA genes was amplified using KAPA2G Robust PCR kit (KAPABiosystems) and

the primers PCR1F_343: (5'-CTT TCC CTA CAC GAC GCT CTT CCG ATC TAC GGR AGG CAG CAG-3') and PCR1R_784: (5'-GGA GTT CAG ACG TGT GCT CTT CCG ATC TTA CCA GGG TAT CTA ATC CT-3'). The PCR mix contained 50 ng of DNA, 1 µl of dNTPs (10 mM), 1.25 µl of each primer (20 µM), and 0.2 µl of Taq polymerase in a total volume of 50 µl. The amplification conditions were: 95°C for 5 min, followed by 40 cycles at 95°C for 30 s, 65°C for 30 s, 72°C for 60 s, and a final extension at 72°C for 10 min. Purified amplicons were sequenced using the MiSeq sequencing technology (Illumina, San Diego, CA, USA) at the GeT-PLaGe platform (Genotoul, Toulouse, France). Paired-end reads obtained from MiSeq sequencing were analyzed using the Galaxy supported pipeline named Find Rapidly Operational Taxonomic Units (OTUs) with Galaxy Solution (FROGS). For the preprocessing, only reads with length ≥380 bp were kept. The clustering and chimera removal steps followed the guidelines of FROGS [27]. Taxonomic assignment was performed using the Silva database (v138, https://www.arb-silva.de/). OTUs with global abundance lower than 0.005 % were removed from the following analysis [28].

Measurement of short-chain fatty acids in caecal contents

SCFA analysis was carried out as described previously [29]. Caecal contents were thawed, water extracted, and proteins were precipitated with phosphotungstic acid. A volume of 0.1 µl supernatant fraction was analysed for SCFA on a gas-liquid chromatograph (Autosystem XL; Perkin Elmer, Saint-Quentin-en-Yvelines, France) equipped with a split-splitless injector, a flame-ionisation detector and a capillary column (15 m x 0.53 mm, 0.5 µm) impregnated with SP 1000 (FSCAP Nukol; Supelco, Saint-Quentin-Fallavier, France). Carrier gas (H2) flow rate was 10 ml.min-1 and inlet, column and detector temperatures were 200°C, 100°C and 240°C, respectively. 2-Ethylbutyrate was used as the internal standard. Samples were analysed in duplicate. Data were collected and peaks integrated using the Turbochrom v6 software (Perkin Elmer, Courtaboeuf, France).

Statistics

Systematically, a normality test and a variance equality test were performed on the data. In case of normal distribution and variance equality, data were expressed as mean ± SEM values obtained from the indicated number of mice, and we used one-way ANOVA, followed by Tukey's multiple comparison test, to compare the three groups of mice. Comparison for sucrose vs. water consumption in the sucrose two-bottle free choice test was done with two-way ANOVA. In case of lack of normal distribution or of variance equality, data were expressed as median (interquartile range) and analyzed using Kruskal-Wallis test, followed by Dunn's multiple comparison test, to compare the three groups of mice. The level chosen for statistical significance was 5 %. All calculations were performed using GraphPad Prism software (version 7.00, La Jolla, CA, USA).

16S rRNA amplicon sequencing bioinformatic analysis was performed on RStudio (v1.1.419) package Phyloseq 1.23.1. Visualizations were performed with the ggplot2 R package and differential analyses were performed with DESeq2 R package. Correction of false discovery rates for multiple tests was performed using the Benjamini-Hochberg method.

Results

The body weight of mice consuming HFD was increased, while their daily food consumption was decreased

Week-15, the weights of HFD-H2O mice (mean, 35.51 ± 1.10 g, n=15) and HFD-AB mice (35.58 ± 0.75 g, n=15) were similar and both groups of HFD mice were significantly heavier (both, $P < 0.001$) than the STDD-H2O mice (29.61 ± 0.29 g, n=15), ($F(2, 42) = 18,8$; $P < 0.001$). The daily food consumption was measured over 12 weeks, between the weeks 2 and 13. The mice on the HFD had a lower food intake (mean, 2.79 ± 0.03 g, n=12) than the mice on the standard diet (3.65 ± 0.03 g, n=12) ($P < 0.001$); the antibiotic treatment did not affect this difference (2.83 ± 0.04 g, n=12) ($P < 0.001$), ($F(2, 33) = 248,5$; $P < 0.001$).

Caecal content weight was differentially modified in HFD-H2O and HFD-AB mice

The caecal content weight in percent of body weight of the STDD-H2O mice was significantly greater ($P < 0.01$) than that of the HFD-H2O mice. In addition, the caecal content weight in percent of body weight of the HFD-AB mice was significantly greater than those of the two other groups (respectively $P < 0.01$ and $P < 0.001$), ($H = 36.7$; $P < 0.001$) (Figure 2).

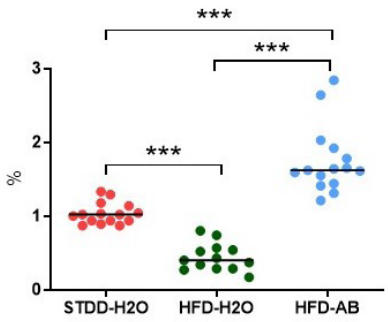


Figure 2. Caecal content weight in percent of body weight. Results are expressed as median, n = 15. *** $P < 0.001$

Locomotor activity was not affected by the HFD diet and the antibiotic treatment

The assessments of the number of squares crossed and rearings did not indicate differences in locomotor activity among the three groups. Similarly, there were no differences between them for the number of grooming and

defecations (Supplementary Table 1).

The HFD consumption induced a degradation of the coat state

Week-15, the coat state scores in the HFD-H2O and HFD-AB groups were significantly higher ($P < 0.001$) respectively in comparison to the score in the STDD-H2O group ($H = 23.7$; $P < 0.001$) (Figure 3).

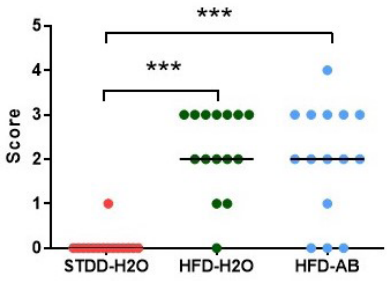


Figure 3. Coat state scores. Results are expressed as median, n = 15. *** $P < 0.001$

Forced swim performance was not affected by the HFD diet and the antibiotic treatment

The duration of immobility was similar among the three groups (Figure 4).

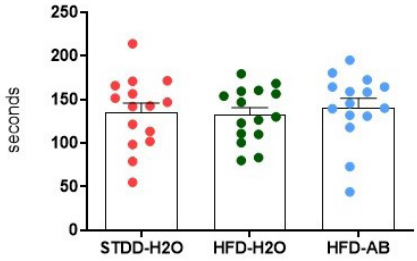


Figure 4. Immobility in the FST. Results are expressed as mean ± SEM, n = 15

The HFD consumption decreased sucrose preference

In the two-bottle-choice drinking paradigm, the total liquid consumption was significantly higher in the STDD-H2O group (mean, 478.1 ± 18.5 ml.kg-1) in comparison with both HFD-H2O (279.5 ± 13.2 ml.kg-1) and HFD-AB (294.8 ± 9.7 ml.kg-1) groups (for both, $P < 0.001$). In the three groups, the mice drank more sucrose solution than water (Figure 5A). However, HFD-H2O and HFD-AB mice exhibited a lower preference for sucrose than the STDD-H2O mice (Figure 5B).

Measurement of cytokines in plasma was not affected by the HFD diet and the antibiotic treatment

Plasmatic levels of the three main pro-inflammatory cytokines, i.e., IL1, IL6 and TNFα showed no significant difference among the three experimental groups of mice (Supplementary Table 2). The cytokines levels in both HFD groups were similar to the values in control mice, suggesting no marked peripheral inflammation whatever the experimental conditions.

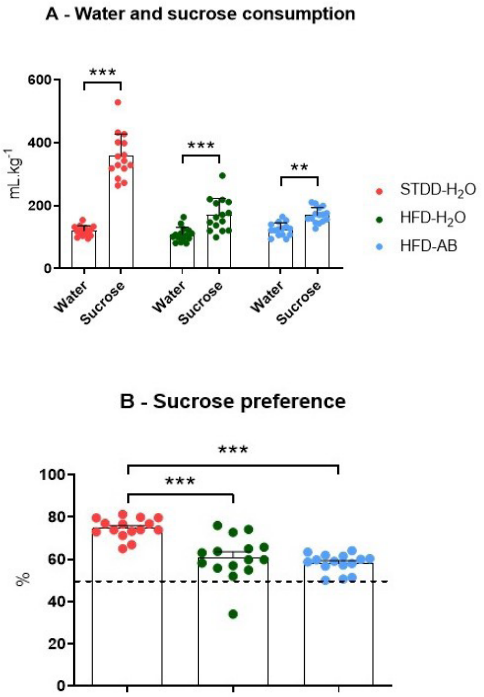


Figure 5. Measurement of water and sucrose consumption in the three groups of mice. (A) Sucrose solution (4%) intake (ml.kg-1) in comparison with water intake. (B) Sucrose preference (%). Results are expressed as mean ± SEM, n = 15. * * $P < 0.01$, * * * $P < 0.001$

Analysis of the 16S rRNA gene sequences showed differences among the three groups of mice

The samples were rarefied to the same sample depth (5607 reads per sample) prior to all diversity analyses. The rarefaction depth chosen was the minimum depth observed among all the samples, which corresponded to a STDD-H2O mouse sample (See supplementary Figure 1). The α-diversity (measured by the Observed, Shannon and InvSimpson indexes) was significantly different between the three groups. We observed a significant decrease in the α-diversity for the three indexes in HFD-H2O mice compared to STDD-H2O mice ($P < 0.001$ for each pairwise comparison). The antibiotic treatment resulted in a fall of the α-diversity in the HFD-AB mice compared to the other two groups of mice ($P < 0.001$ for each pairwise comparison) (Figure 6A). Beta diversities were computed on all samples using the Bray-Curtis distance and the resulting pairwise distance matrix was used to perform a principal coordinate analysis (PcoA). The PCoA plot shows that samples cluster by group, and that the groups are well separated (Permutational multivariate ANOVA with adonis, $P < 0.001$) (Figure 6B). This separation was also observed when considering the Jaccard distance (not

shown).

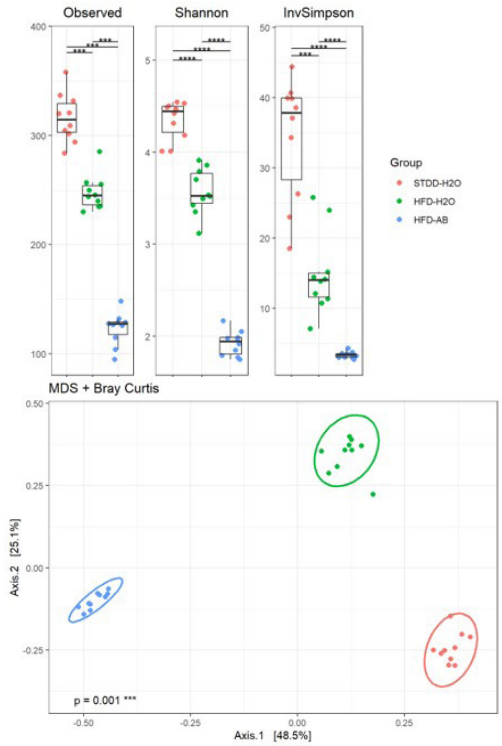
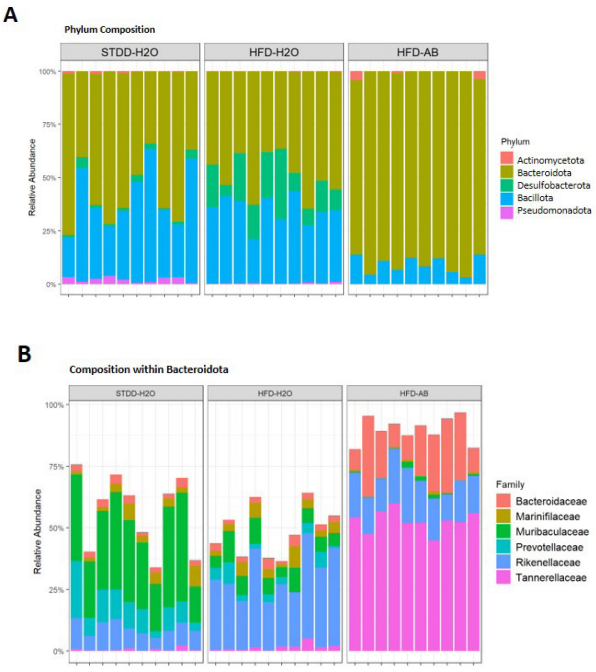


Figure 6. Gut microbiota composition differed in the three group of mice. Analysis was based on 16S rRNA gene sequencing. (A) The box-plot figures show the alpha diversity of the bacterial communities in the three groups STDD-H2O, HFD-H2O and HFD-AB by means of Observed, Shannon, and Inverse Simpson indexes (n = 10; ** P < 0.01, *** P < 0.001, **** P < 0.0001). (B) PCoA ordination plot of the Bray Curtis distance matrix between samples show the beta diversity indicating the separation of the three bacterial communities (n = 10).

Similarly, phylum relative abundances and, within the phyla, family relative abundances differed between the three groups (Figure 7). The change caused in the HFD-H2O mice compared to STDD-H2O mice at the phyla level was an increase in the relative abundance of Desulfobacterota ($P < 0.01$) and a decrease in the relative abundance of Pseudomonadota ($P < 0.05$), with no change in Bacteroidota and Bacillota (Figure 7A). When the main family profiles were examined within each phylum, we observed that the HFD leads to strong changes in relative abundance in each of them in the HFD-H2O mice compared to STDD-H2O mice: in Bacteroidota, decreased Muribaculaceae ($P < 0.01$) and Prevotellaceae ($P < 0.05$), and increased Rikenellaceae ($P < 0.001$) (Figure 7B); in Bacillota, decreased Butyricicoccaceae ($P < 0.01$), and increased Erysipelotrichaceae ($P < 0.05$), Oscillospiraceae ($P < 0.05$), Peptostreptococcaceae ($P < 0.001$) and Streptococcaceae ($P < 0.001$) (Figure 7C); in Actinomycetota, decreased Bifidobacteriaceae ($P < 0.001$) (Figure 7D); in Desulfobacterota, increased Desulfovibrionaceae ($P < 0.01$) (Figure 7E); in Pseudomonadota, decreased Sutterellaceae ($P < 0.01$) and increased Enterobacteriaceae ($P < 0.01$) (Figure 7F).

Antibiotic treatment resulted in a strong change in the composition of the gut microbiota, both in comparison to the STDD-H2O and HFD-H2O mice. It was characterised by a predominant abundance of Bacteroidota ($P < 0.001$ for both comparisons), a reduction in Bacillota ($P < 0.001$ for both comparisons) and a nearly complete disappearance of Desulfobacterota (Respectively $P < 0.01$ and $P < 0.001$) and Pseudomonadota (Respectively $P < 0.001$ and $P < 0.01$) (Figure 7A). Regarding the differences within each phylum, in the HFD-AB group, the family profile was strongly affected by the antibiotic treatment, with changes in abundance for several families in comparaison with those in mice of the HFD-H2O group: in Bacteroidota, decreased Muribaculaceae ($P < 0.01$), Prevotellaceae ($P < 0.01$) and Rikenellaceae ($P < 0.05$), and increased Bacteroidaceae ($P < 0.001$) and Tannerellaceae ($P < 0.01$) (Figure 7B); in Bacillota, decreased Butyricicoccaceae ($P < 0.01$), Clostridiaceae ($P < 0.001$), Lachnospiraceae ($P < 0.01$), Lactobacillaceae ($P < 0.001$), Oscillospiraceae ($P < 0.001$), Peptostreptococcaceae ($P < 0.001$), and Ruminococcaceae ($P < 0.001$), and increased Christensenellaceae ($P < 0.05$) (Figure 7C); in Actinomycetota, decreased Eggerthellaceae ($P < 0.001$) (Figure 7D); in Desulfobacterota, decreased Desulfovibrionaceae ($P < 0.001$) (Figure 7E); in Pseudomonadota, decreased Enterobacteriaceae ($P < 0.001$) and Sutterellaceae ($P < 0.05$) (Figure 7F).



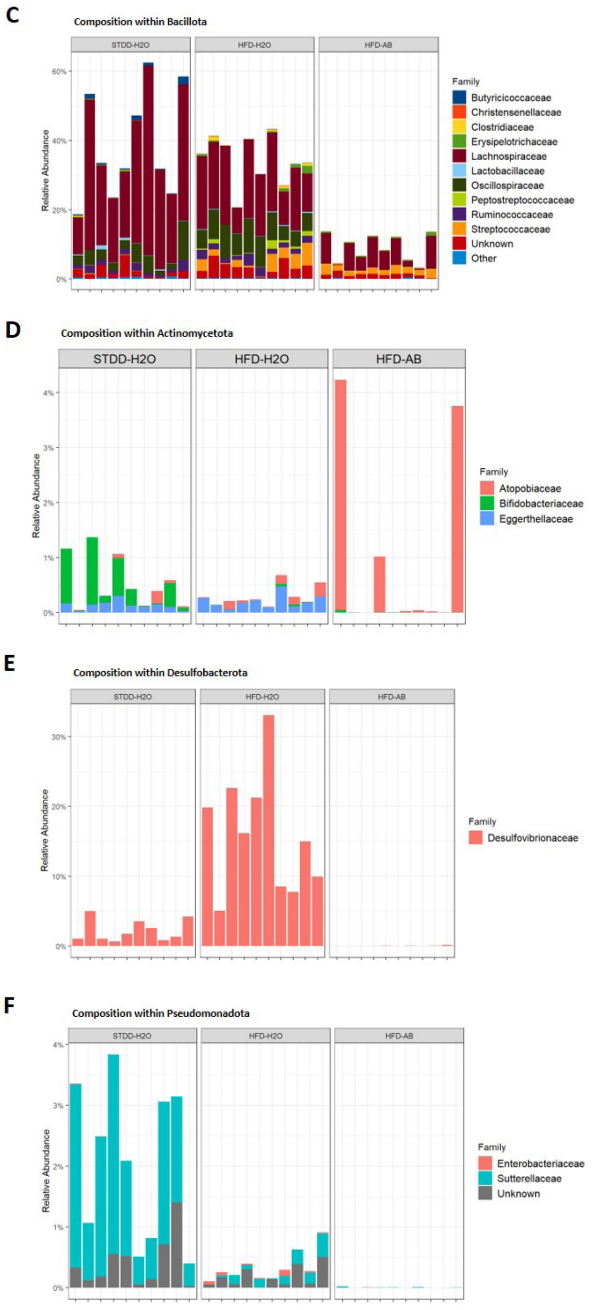


Figure 7. Bar graph shows the relative abundance of phyla (A) and of most abundant families within the phyla (B–F) in the three groups of mice STDD-H2O, HFD-H2O and HFD-AB (n = 10)

For only a few families, the relative abundance which was modified by the HFD was not further affected by the antibiotic treatment. This was the case in the Bacillota, for the Streptococcaceae and the Erysipelotrichaceae and in the Actinomycetota, for the Bifidobacteriaceae. In the Bacteroidota, a family, the Rikenellaceae, showed the same response profile despite a significant difference between the HFD-H2O and HFD-AB groups (P<0.05) (Figure 8).

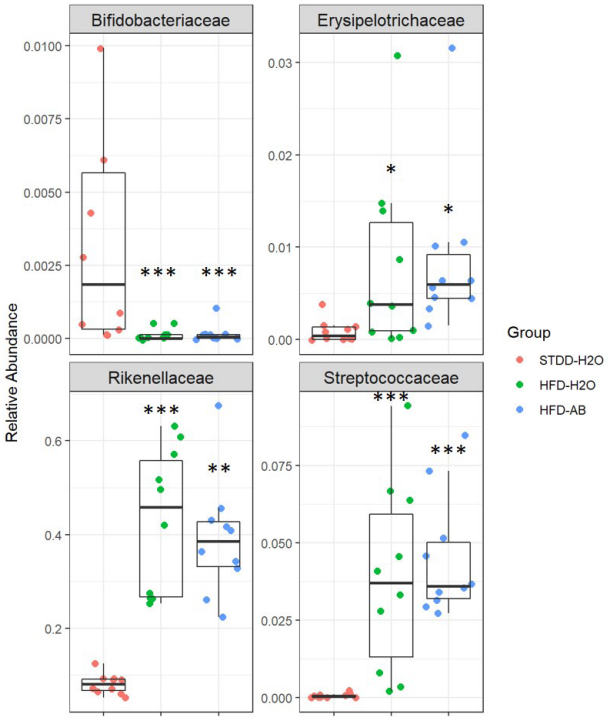


Figure 8. The box-plot figures show the relative abundance of the four families that are modified by HFD but not by the antibiotic treatment. Results are expressed as median (interquartile range), n = 10. (n = 10). * P < 0.05, ** P < 0.01, *** P < 0.001 relative to the STDD-H2O group

SCFAs in caecal contents were differentially modified in the three groups of mice

The HFD-H2O mice exhibited the same SCFA concentration expressed in µmol/g caecal content (median, 25.04(10.69)) as the STDD-H2O mice (32.35(22.56)) (Supplementary figure 2). The SCFA concentration in HFD-AB mice (5.52(2.58)) was dramatically decreased in comparison with those of the two other groups of mice (both P < 0.001) (H = 19.8; P < 0.001). The concentrations of acetate, propionate, butyrate and long- and branched-chain fatty acids were globally different between groups (respectively (H = 22.3, P < 0.001; H = 19.0, P < 0.001; H = 18.9, P < 0.001; H = 9.9, P < 0.01). The concentration of acetate in STDD-H2O mice (15.05(9.72)) and in HFD-H2O mice (17.25(5.68)) was significantly higher than in HFD-AB (3.55 (1.51)) (both P < 0.001). On the other hand, if we compared the proportions in comparison with the total SCFA, we observed that the proportion of acetate in HFD-H2O mice (67.82(6.67) %) and in HFD-AB mice (63.89(22.08) %) was significantly higher than in STDD-H2O mice (53.30(9.99) %) (both P < 0.05). The concentration of propionate in STDD-H2O mice (4.15(2.70)) and in HFD-H2O mice (3.06(4.86)) was significantly higher than in HFD-AB (1.18 (0.31)) (both P < 0.001). In contrast, there was no difference in the proportion of propionate between the three groups. The concentration of butyrate in STDD-H2O mice

(9.19(10.61)) and in HFD-H2O mice (2.72(1.43)) was significantly higher than in HFD-AB (0.28 (0.30)) (both $P < 0.001$). In the same way, the proportion of butyrate in STDD-H2O mice (26.78(19.48) %) and in HFD-H2O mice (10.82(3.82) %) was higher than in HFD-AB mice (5.45(1.89) %) (respectively, $P < 0.001$ and $P < 0.05$). The concentration of long- and branched-chain fatty acids in HFD-H2O mice (1.52(0.63)) was higher than in STDD-H2O mice (0.90(0.33)) ($P < 0.05$). In the same way, the proportion of long- and branched-chain fatty acids in HFD-H2O mice (5.80(1.17) %) was higher than in STDD-H2O mice (3.33(2.28) %) ($P < 0.01$). Otherwise, the concentration of long-and-branched-chain fatty acids in HFD-AB mice (0.27(0.26) %) was smaller than in STDD-H2O and in HFD-H2O mice (respectively, $P < 0.001$ and $P < 0.01$). (Supplementary figure 2).

Discussion

In this study, we found that the HFD-induced increase in body weight in male mice was accompanied by substantial changes in the composition of the gut microbiota and in two markers of depressive-like behaviour, altered coat condition and anhedonia. In addition, in DIO mice, antibiotic treatment led to very marked alteration in the composition of the gut microbiota without any change in depression-like behaviour.

The consumption of HFD caused an increase in the weight of the mice as expected, of the same level as previously described [30-31]. Among the indexes of depression we considered after the 14 to 15 weeks of HFD, we did not observe change in helplessness in the forced swim test, but an impaired coat state and a reduced preference for sucrose. To our knowledge the coat state score that is used to report a depressive-like condition after chronic mild stress in mice [23], has never been used in DIO mice. For the other two indexes, our results are in total accordance with those obtained with C57BL/6J mice by Takase et al. [5] who used the same HFD as ours.

In several studies conducted in C57BL/6 mice, using the same HFD or a HFD with 60 kcal% from fat during at least 3 months, no helplessness was induced in the forced swim test [5,32-34]. Three studies in C57BL/6 mice using more concentrated HFD (50 to 60 kcal% from fat) showed helplessness in the forced swim test [35-37]). In addition, in the opposite of our results, Hasebe et al. [38], using a HFD with 45 kcal% from fat, observed helplessness in the forced swim test but no anhedonia in the sucrose preference test. Several studies have observed an induction of anhedonia as we did, but after a HFD with a greater fat concentration than the one we used [21, 32, 39-42]. These studies indicated that the induction of depressive-like behaviours in DIO mice is present in the majority of studies, but may vary according to experimental conditions. In our study only anhedonia was present in mice from the HFD-H2O group, but this is an important indicator since the reduced ability to experience hedonic pleasure in response to previously rewarding stimuli is a core symptom of major depressive disorder [43].

The 16S rRNA amplicon sequencing revealed, through the α-diversity and diversity indexes, that the gut microbiota composition was significantly altered after 15 weeks of HFD. In terms of phylum composition, this was particularly reflected by an increase in the relative abundance of the Pseudomonadota phylum in the HFD-H2O group in comparison to the STDD-H2O group. The increased abundance of Pseudomonadota has been described as a potential diagnostic signature of dysbiosis and risk of disease, and several studies have shown an increase in its relative abundance in C57BL/6J mice following HFD (for review, see [44-45]). We did not observe the reduced abundance of Bacteroidota and proportional increase in Bacillota, as well as the resulting increase in the Bacillota/Bacteroidota ratio (data not shown), usually associated with obesity in humans and DIO rodents (for a review see [38,46]). However, this association, as a characteristic of obesity, is questioned in a recent review, which shows that it was not observed in all studies, some even describing a decrease in the Bacillota/Bacteroidota ratio [47].

Regarding the SCFA, the HFD did not modify the overall SCFA caecal concentration, and the concentrations of acetate, propionate and butyrate in HFD-H2O mice in comparison to STDD-H2O mice. In contrast, the HFD induced an increase of long and branched chain fatty acids concentration, as well as an increase in long and branched chain fatty acids and acetate proportions that were concomitant with the induction of anhedonia by the HFD. The possibility that the gut microbiota and its SCFA metabolites play a role in the induction of anhedonia was raised in a recent article, but it focused on butyrate [48].

The dramatic change in the composition of the gut microbiota induced by antibiotic treatment in HFD-AB mice compared to mice from the STDD-H2O and HFD-H2O groups results in a predominant abundance of Bacteroidota, a reduction in Bacillota and a nearly complete disappearance of Desulfobacterota and Pseudomonadota. These changes in the proportion of the four phyla are consistent with what is observed in studies in which reversion to obesity occurs (for review, see [38,45-46]), but the 3 weeks duration of the antibiotic treatment following 12 weeks of HFD probably did not allow this result to be materialized by weight loss. Several studies have shown a reduction in weight gain following antibiotic treatment, but in these studies the treatment was administered over the duration of the HFD for 4 to 12 weeks [21,49-50]. In addition, previous experiments in which the gut microbiota of DIO mice treated with the same combination of antibiotics as in this study was transferred to germ-free mice subsequently fed on a HFD showed that their body weight and food intake were the same as those of germ-free mice receiving microbiota of

control mice and fed on a HFD [51].

In the present study, the three weeks antibiotic treatment at the end of the HFD did not result in any change in anhedonia, helplessness and coat state in the HFD-AB mice compared to the HFD-H2O mice. The literature shows that there is dysbiosis in patients suffering from depression and that it has been possible, in preclinical studies, to transfer depressive-type behaviour to mice by faecal transplantation [11-17]. Since in this study, DIO mice exhibit depression-like behaviour, the composition of their microbiota could be a factor favouring depression, so a significant change in the composition of the gut microbiota following antibiotic treatment could be expected to alter this ability. This is the case in the only study that has examined the effect of antibiotic treatment in DIO mice. In this study, in parallel with a 4-week HFD diet, the use of an antibiotic treatment that has severely depleted gut bacteria and modified gut microbiota composition, was accompanied by a correction of anhedonia [21]. The discrepancy in the impact of the treatments on anhedonia between this study, and ours, could be due to differences in the proportions of bacterial families affected by each antibiotic treatment. Unfortunately, the study by Hassan et al. [21], only indicated the overall effects on the gut microbiota, without mentioning the consequences at the level of the individual taxa. In addition, it should be noted that dysbiosis induced by antibiotic treatment in healthy C57BL/6 mice leads to contradictory results with regard to depressive-like behaviours, since treatment with broad spectrum antibiotic cocktails resulted either in a decrease in depressive-like behaviour [19] or increased depressive-like behaviour in the forced swim test and/or tail suspension test [18,20].

The antibiotic treatment decreases the overall SCFA caecal concentration, as well as the concentration of all the different SCFAs individually, in the HFD-AB mice in comparison with the STDD-H2O and HFD-H2O mice, which is consistent with the marked change in the composition of the gut microbiota. The treatment has no effect on the increase of acetate proportion induced by the HFD, which were similar in HFD-H2O and HFD-AB groups; in contrast it induced a decrease of the butyrate proportion, in the HFD-AB group in comparison with the STDD-H2O and HFD-H2O groups, which suggests a specific reduction of the butyrate-producing bacterial species. This result is consistent with the strong decrease that we observed in Bacillota, the main butyrate producing phylum.

When we looked at the evolution of the main family profiles within each phylum, we noticed that in HFD-H2O mice, HFD consumption led to a modification in the relative abundance of 12 bacterial families. The antibiotic treatment resulted in a marked alteration in the relative abundance of bacterial families in HFD-AB mice, and this concerned 8 of the 12 families profiles previously affected by HFD consumption. The four families for which the relative abundance was not affected, were in the Bacteroidota, the Rikenellaceae, in the Bacillota, the Streptococcaceae and the Erysipelotrichaceae and in the Actinomycetota, the Bifidobacteriaceae. In the growing evidence that the gut microbiota is involved in obesity-related depression, this maintenance in HFD-AB mice, parallel to the non-evolution of anhedonia suggests that these families may be involved in this depression-like behaviour.

Interestingly, the bacterial families we have identified are among the bacterial families described in a recent review and meta-analysis which included 59 case-control studies assessing the diversity and abundance of gut microbiota in a general adult population with psychiatric disorders (such as major depressive disorder, bipolar disorder, psychosis and schizophrenia, anorexia nervosa, anxiety, obsessive compulsive disorder, posttraumatic stress disorder, or attention-deficit/hyperactivity disorder) [52]. Fifty-seven of 59 identified significant differences between patients and control at phylum, family, or genus level. Twenty-one were focused on major depressive disorder and revealed 25 families differentially abundant. Among them are the Bifidobacteriaceae, the Rikenellaceae and the Streptococcaceae. The four studies showing a difference in Bifidobacteriaceae relative abundance were all consistent and reported its increase, in contrast with the decrease in relative abundance of Bifidobacteriaceae that we observed in both HFD-H2O and HFD-AB mice. For the Rikenellaceae and Streptococcaceae families, the changes in relative abundance, observed in 2 and 3 studies respectively, were contradictory and did not allow a trend to be determined. While Erysipelotrichaceae was not part of the families whose relative abundance was altered, the authors of the review indicated that at the genus level, 3 out of 4 studies reported an increase in the relative abundance of an Erysipelotrichaceae incertae sedis bacterium. What emerges more generally from this review is that in the current state of knowledge it is not possible to link specific taxa to psychiatric pathologies, as the results are not yet consistent. However, results at different taxonomic levels highlight interesting families for future interventional studies.

We noted some limitations to our study. In addition to the dramatic change in the composition of the gut microbiota, the antibiotic treatment may have resulted in a reduction of the overall bacterial load. Although this data was not assessed in the present study, the decrease in the concentration of the different SCFAs could be an indicator of its occurrence. It may be noted that while the decrease in SCFAs concentrations was substantial, their concentrations remained at quantifiable levels, and the ratio of acetate to butyrate and propionate around 3:1:1 observed in controls was maintained. This indicates that even if the overall bacterial load was reduced, a functional bacterial ecosystem in the gut was nevertheless still present. The four bacterial families whose abundances were unaffected by the antibiotic treatment in DIO mice may have been in lower absolute levels compared to the

untreated groups; however, in this bacterial ecosystem modified by antibiotic treatment, the maintenance of their relative abundance may reflect the maintenance of their activities and their relationships within the bacterial ecosystem and with the host. Another point, we only used male mice and it is known that depression has a sex-dependent effect, with greater prevalence among females. As a future step, we will have to ascertain whether similar or amplified results can be observed in female mice. We chose to perform the study on C57BL/6J mice, but it is possible that the more emotive strains, such as BALB/c mice, would have produced more significant results. Indeed, it is possible that the results we are observing are the consequence of a complex interplay between genetic background and gut microbiota [53]. Therefore, the four bacterial families that we have identified are possibly present in a particular context linked to the sex and strain chosen. In the future, only the multiplication of preclinical and clinical studies will allow the cross-checking of taxa of interest in the physiopathology of the obesity-depression comorbidity. In addition, rather than the involvement of specific taxa, perhaps it is the combination of bacteria creating a particular ecological context and a particular bacterial metabolism that could participate in the emergence of this comorbidity.

Conclusion

Our study demonstrated that consumption of a high-fat diet resulted in significant changes in both behaviour and gut microbial composition. Our study also demonstrated that antibiotic treatment significantly altered the gut microbial profiles of DIO mice, without affecting in our experimental condition the coat state and anhedonia behaviour. Four families of bacteria, Bifidobacteriaceae, Erysipelotrichaceae, Rikenellaceae and Streptococcaceae, emerged from this study as potential biomarkers of obesity-depression interaction.

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Authors' contribution

LN, NC, SR, SV designed and performed experiments, analysed data and wrote the manuscript; CP, MM, PF performed experiments and analysed data; all the authors reviewed the manuscript.

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Conflict of interest

The authors declare no competing interests.

Consent for publication

All authors have approved the manuscript and agreed with its submission to the Journal of Food, Nutrition and Diet Science

Supplementary materials

The supplementary figures and tables mentioned in above are available at
https://file.luminescience.cn/FNDS-172-Supplementary%20material.zip

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