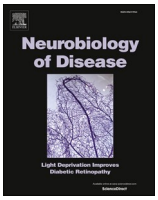
Neurobiology of Disease 159 (2021) 105495

Contents lists available at ScienceDirect

Neurobiology of Disease

journal homepage: www.elsevier.com/locate/ynbdi



High-fat diet alters stress behavior, inflammatory parameters and gut microbiota in Tg APP mice in a sex-specific manner

Natalia Yanguas-Casás [a,b,c,1], Cristina Torres-Fuentes [d,e,f,1], Andrea Crespo-Castrillo [a], Sonia Diaz-Pacheco [a], Kiera Healy [d,g], Catherine Stanton [d,g], Julie A. Chowen [h,i,j], Luis M. Garcia-Segura [a,b], Maria Angeles Arevalo [a,b], John F. Cryan [d,e], Maria L. de Ceballos [a,*]

[a] Cajal Institute, CSIC, 28002 Madrid, Spain
[b] Centre for Biomedical Network Research for Frailty and Healthy Ageing (CIBERFES) Instituto de Salud Carlos III, Madrid, Spain
[c] Lymphoma Research Group, Medical Oncology Department, Instituto de Investigación Sanitaria Puerta de Hierro-Segovia de Arana, Majadahonda, Madrid, Spain
[d] Dept Anatomy & Neuroscience, University College Cork, Cork, Ireland
[e] APC Microbiome Ireland, University College Cork, Cork, Ireland
[f] Universitat Rovira i Virgili, Biochemistry and Biotechnology Department, 43007 Tarragona, Spain
[g] Teagasc Food Research Centre, Moorepark, Fermoy, Cork, Ireland
[h] Department of Endocrinology, Hospital Infantil Universitario Niño Jesús, Instituto de Investigación la Princesa, 28009 Madrid, Spain
[i] Centre for Biomedical Network Research for Physiopathology of Obesity and Nutrition (CIBEROBN), Instituto de Salud Carlos III, Madrid, Spain
[j] The Madrid Institute for the advanced study of Food (IMDEA de Alimentación), Madrid, Spain

ARTICLE INFO

Keywords:
Anxiety
Alzheimer's disease
Cytokines
Diet
Inflammation
Microbiota
Microglia
Sex differences
Phagocytosis
TgAPP mice

ABSTRACT

Long-term high-fat diet (HFD) consumption commonly leads to obesity, a major health concern of western societies and a risk factor for Alzheimer's disease (AD). Both conditions present glial activation and inflammation and show sex differences in their incidence, clinical manifestation, and disease course. HFD intake has an important impact on gut microbiota, the bacteria present in the gut, and microbiota dysbiosis is associated with inflammation and certain mental disorders such as anxiety. In this study, we have analyzed the effects of a prolonged (18 weeks, starting at 7 months of age) HFD on male and female mice, both wild type (WT) and TgAPP mice, a model for AD, investigating the behavioral profile, gut microbiota composition and inflammatory/phagocytosis-related gene expression in hippocampus. In the open-field test, no overt differences in motor activity were observed between male and female or WT and TgAPP mice on a low-fat diet (LFD). However, HFD induced anxiety, as judged by decreased motor activity and increased time in the margins in the open-field, and a trend towards increased immobility time in the tail suspension test, with increased defecation. Intriguingly, female TgAPP mice on HFD showed less immobility and defecation compared to female WT mice on HFD. HFD induced dysbiosis of gut microbiota, resulting in reduced microbiota diversity and abundance compared with LFD fed mice, with some significant differences due to sex and little effect of genotype. Gene expression of pro-inflammatory/phagocytic markers in the hippocampus were not different between male and female WT mice, and in TgAPP mice of both sexes, some cytokines (IL-6 and IFNγ) were higher than in WT mice on LFD, more so in female TgAPP (IL-6). HFD induced few alterations in mRNA expression of inflammatory/phagocytosis-related genes in male mice, whether WT (IL-1β, MHCII), or TgAPP (IL-6). However, in female TgAPP, altered gene expression returned towards control levels following prolonged HFD (IL-6, IL-12β, TNFα, CD36, IRAK4, PYRY6). In summary, we demonstrate that HFD induces anxiogenic symptoms, marked alterations in gut microbiota, and increased expression of inflammatory genes, except for female TgAPP that appear to be resistant to the diet effects. Lifestyle interventions should be introduced to prevent AD onset or exacerbation by reducing inflammation and its associated symptoms; however, our results suggest that the eventual goal of developing prevention and treatment strategies should take sex into consideration.

Abbreviations: AD, Alzheimer's disease; APP, Amyloid precursor protein; HFD, high-fat diet; LFD, low fat diet; LPS, lipopolyscharide; OTU, operational taxonomic units; PCoA, principal co-ordinate analysis; Tg, transgenic; TST, tail suspension test;; WT, wild type;.

* Corresponding author at: Cajal Institute, CSIC, Av. Doctor Arce, 37, 28002 Madrid, Spain.
E-mail address: mceballos@cajal.csic.es (M.L. de Ceballos).

[1] These authors equally contributed.

https://doi.org/10.1016/j.nbd.2021.105495
Received 17 May 2021; Received in revised form 13 August 2021; Accepted 27 August 2021
Available online 31 August 2021

1. Introduction

Alzheimer's disease (AD), the major cause of dementia, is characterized by β-amyloid (Aβ) plaques and neurofibrillary tangles, the loss of particular subsets of neurons, and glial activation. In fact, one invariant feature of AD is the existence of neuroinflammation. Neuroinflammation is a potent contributor to both the initiation and progression of AD. Indeed, its neuropathology is characterized by a sustained innate immune response that includes the presence of activated microglia and production of inflammatory cytokines that are favored by the generation of soluble Aβ (Akiyama et al., 2000; Dickson et al., 1993; Heppner et al., 2015). In recent years, neuroinflammation per se has been postulated to be a causal component of AD, rather than a consequence of neurodegeneration, and has been shown to correlate better with cognitive decline than does Aβ accumulation (Heneka et al., 2015; Holmes et al., 2009; Perez-Nievas et al., 2013; Wyss-Coray, 2006). Interestingly, systemic inflammation, promoted by peripheral infection, may interact with the innate immune response of the brain, acting as the driver of disease progression and exacerbating AD symptoms (Heneka et al., 2015; Holmes et al., 2009, 2011). Moreover, the fact that prolonged treatment with anti-inflammatory drugs appears to decrease the risk of suffering AD (Int Veld et al., 2001; McGeer et al., 1996) suggests that prevention of the neurologic illness with anti-inflammatory therapies may be feasible. However, prospective clinical trials with a variety of NSAIDs of patients diagnosed with AD have demonstrated a lack of efficacy (Cuello, 2017; Heneka et al., 2015).

Interestingly, there is a striking sex bias in the incidence, clinical manifestation, disease course, and prognosis of AD, with females being more vulnerable than males (Carr et al., 1997; Pike, 2017), a sex difference that likely results from sex steroid hormone effects (Paganini-Hill and Henderson, 1994; Pike et al., 2009). Indeed, in transgenic (Tg) mouse models of AD, females show earlier Aβ deposition and cognitive deficits than males (Callahan et al., 2001). Furthermore, castration exacerbates both cognitive impairment and Aβ pathology (Carroll et al., 2007), while estrogen replacement therapy exerts beneficial effects (Carroll et al., 2007; Zhao et al., 2013), more so when timely initiated at the onset of menopause (Whitmer et al., 2011; Zhao et al., 2013). Besides sex hormones, other reasons should be taken into account as well. Indeed genetic factors, such as ApoE or BDNF genetic variants, increased immune responses in females, and their susceptibility to stress-influenced diseases may confer predisposition for AD (Fisher et al., 2018).

Two types of risk factors contribute to the development of AD: non-modifiable risk factors, such as genetic susceptibility or sex, and modifiable risk factors that are tightly related to neuroinflammation, which includes health- and lifestyle-related factors that can be altered by changing individual behaviors (Cosimo Melcangi and Garcia-Segura, 2010; McKenzie et al., 2017). Among the latter, obesity has been postulated as a risk factor for cognitive decline and AD (Smith et al., 2011), possibly as a result of the increased serum levels of pro-inflammatory mediators that lead to chronic systemic inflammation, eventually triggering neuroinflammation, exacerbating Aβ-induced degenerative cascades, and favoring AD progression (Guillemot-Legris and Muccioli, 2017; Heneka et al., 2015).

Obesity is one of the most important health problems that developed countries are facing (Malik et al., 2013). The propensity to gain weight or become obese differs between men and women, and men have a higher incidence of obesity-associated health complications (Nedungadi and Clegg, 2009; Power and Schulkin, 2008; Tramunt et al., 2020). Similarly, sex is an important factor in weight gain and metabolic consequences of diet in rodents (Ingvorsen et al., 2017; Zore et al., 2018). The same dietary factors that lead to obesity (e.g., excess consumption of a high carbohydrate/HFD) also impair cognitive functioning. The hippocampus is a key center for cognition, and HFD increases hippocampal inflammation and augments the permeability of the blood–brain-barrier leading to the accumulation of exogenous substances and causing deficits in hippocampal-dependent memory (Martin and Davidson, 2014). A 27-year longitudinal population-based study indicates that midlife obesity raises the risk of future dementia (Kivipelto et al., 2005; Pasinetti and Eberstein, 2008), and accumulating evidence suggests that cognitive dysfunction should be prominent on the list of the adverse health consequences of living in an obesogenic environment (Martin and Davidson, 2014). In normal rodents, HFD induces alterations in learning and memory tasks, while in some cases pathological features characteristic of AD occurs (Biyong et al., 2021; Hwang et al., 2010; Kim et al., 2016; Leigh et al., 2020), in particular in male mice. Similar detrimental effects of HFD consumption have been reported in transgenic models of AD. For instance, prolonged HFD or sucrose intake increases amyloid and tau pathologies (Cao et al., 2007; Fitz et al., 2010; Julien et al., 2010) and aggravates the cognitive deficits of different transgenic AD mice. In other studies, 5 months of HFD increased memory impairment and Aβ deposition in Tg APP mice (Maesako et al., 2012a, 2012b) that were ameliorated by environmental enrichment, exercise, or diet control. However, Knight et al. (2014) found that cognitive impairment induced by HFD was independent of amyloid and tau pathology changes. Sex appears to be important in the consequences of an HFD as Barron et al. (2013) report ectopic fat accumulation, hyperglycemia, and hyperinsulinemia were observed only in 3xTg-AD male mice, while both male and female mice exhibited significant worsening in behavioral performance and increased hippocampal accumulation of Aβ.

Gut microbiota, the commensal bacteria present in the gut, has emerged as another source of inflammatory mediators (Fülling et al., 2020; Leigh et al., 2020), both in the periphery and central nervous system (CNS). The so-called gut-brain axis provides reciprocal homeostatic communication through immunological, hormonal, and neuronal signals (Burcelin, 2016) and modifies brain and behavior, including recognition memory (Cryan and Dinan, 2012; Leigh et al., 2020). The impact of diet on microbiota composition is well known (Murphy et al., 2010; Morrison et al., 2020; Wu et al., 2011), and HFD reduces microbiota diversity, changes the abundance of different bacteria, and increases intestinal permeability, favoring endotoxin (LPS: lipopolysaccharide) release into the blood (Cani et al., 2007). Importantly, the contribution of HFD-induced dysbiosis on disease risk (e.g. cancer, diabetes, cardiovascular or central nervous system diseases) has been recognized, and recent evidence provides a compelling argument to include gut microbiota as another potential player (Murphy et al., 2015).

Given the higher risk for AD of individuals suffering from metabolic syndrome induced by HFD, the importance of activated microglia in AD (see review by Heneka et al., 2015) and that microbiota controls the maturation and function of microglia (Erny et al., 2015), the gut microbiota has been studied in transgenic models of AD and patients afflicted by the disease (Cryan et al., 2020). For instance, Harach et al. (2017) compared Tg APP/PS1 with control animals, reporting major age-related shifts in the gut microbiota composition, both at the phylum and genus levels. Interestingly, amyloidosis appears to be regulated by the host microbiota of Tg APP/PS1, since antibiotic treatment or germ-free raising conditions induced a reduction in Aβ pathology (Minter et al., 2017; Harach et al., 2017). On the other hand, there is evidence that microbiota in AD patients is altered (Cattaneo et al., 2017), and the changes were correlated with cerebrospinal fluid AD biomarkers as reported in another study (Vogt et al., 2017).

Taking into account that HFD has a large impact on microbiota composition, that microbiota dysbiosis may be another source of inflammatory mediators that could aggravate neuroinflammation and behavioral alterations in AD, and that the consequences of HFD are sex-dependent, we sought to determine the interaction of diet and sex in normal and AD brain. Indeed there is lack of studies investigating the impact of HFD-induced gut microbiota dysbiosis on AD features, such as neuroinflammation or anxiety-related behavior. Furthermore, the comparison of both sexes is of special relevance, since previous studies have used mice of one sex, and more recently groups mixing both sexes.

To this aim, we have studied the effect of diet on cytokine expression in the hippocampus, gut microbiota composition, and diversity and anxiety traits.

2. Material and methods

2.1. Animals

Male and female transgenic APP (Tg APP, line 2576) mice were raised in our in-house colony (Instituto Cajal, CSIC). Transgenic mice along their control WT littermates were housed in the same cages (n = 4–6 per cage) and maintained on chow and water ad libitum in a 12 h dark-light cycle, at a constant temperature (21 ± 1 °C) and humidity (50 ± 1%). Animal handling and care were performed in compliance with the ARRIVE guidelines, the European Union guidelines 2010/63/EU and the Spanish regulations (BOE67/8509–12; BOE 1201/2005) regarding the use and care of laboratory animals, and the protocols were approved by the local Animal Care and Ethics Committee of the CSIC.

2.2. HFD/LFD protocol in WT and TgAPP transgenic mice

A total of 18 TgAPP transgenic male mice, 18 TgAPP transgenic female mice, and 10 age-matched WT control littermates per sex were used in this experimental approach. Animals were genotyped by PCR from tail biopsies. Until 7–8 months of age all mice were fed with standard chow (3.96 Kcal/g, 5.3% fat; 18,9% protein; 3.9% fiber; soya free; plus aas and vitamins, Rod18-H, Altromin). Half of the mice were fed a high-fat diet (HFD; 5.1 Kcal/g, 61.6% kcal from fat, 18% kcal from proteins, 20% kcal from carbohydrates, LabDiet, Sodispan Research SL, Madrid, Spain) or a low-fat diet (LFD; 3.76 Kcal/g, 10.2% kcal from fat, 18% kcal from proteins, 72% kcal from carbohydrates; LabDiet) ad libitum for 18 weeks, starting at 7–8 months of age. Mice were weighed weekly from the beginning of the defined high- or low-fat diet administration to the end of the experimental procedure. After this period, animals were sacrificed by decapitation, their brains were removed. The hippocampi quickly dissected on a cold plate, stored at −80 °C, and then processed to study gene expression by quantitative PCR (qPCR).

HFD increased body weight in female mice (suppl. Table 1), regardless of their genotype. On the HFD, the weight of male TgAPP mice was significantly greater, but not that of WT males, at the time of sacrifice. The weight of diet consumed was recorded twice a week for each cage, averaged and expressed as daily calorie intake (Kcal/day/mouse). Average daily calorie intake just before sacrifice (suppl. Table 1) was similar in LFD and HFD male mice, but females on the HFD had a higher daily calorie intake compared to female mice on the LFD. However, as both WT and TgAPP littermates were housed in the same cage the food intake was not calculated according to genotype.

2.3. Behavioral tests

All of the behavioral experiments were conducted at the same time of the day (9:00 h to14:00 h). The procedures were performed by experienced personnel, who were unaware of the treatments/conditions, although the increased weight was visually obvious when it occurred. Motor activity was monitored in 10 activity cages (Digiscan; AccuScan Instruments, Columbus, OH), equipped with horizontal and vertical photo beam sensors, in an isolated room for 10 min. Horizontal motor activity, number of rearings, and time spent in the margins of the arena were analyzed. At the end of the session, the fecal pellets of each animal were counted.

Finally, the animals were subjected to a tail suspension test (TST) to assess possible stress/depressive behavior. In this test, the mice were suspended by the tail 25 cm above the floor so that their bodies dangled in the air, facing downward for 6 min. The behavior of the animals was recorded with a camera (JVC Mod QZ MG 20), transferring the video clips directly to a PC for analysis. Mice attempted to escape or remained motionless, hanging passively. The data is presented as immobility time.

2.4. Microbial DNA extraction, amplification, and high throughput DNA sequencing

Caecal samples were obtained at sacrifice, after flushing dissected colons with PBS, blotted dry, and were frozen (−80 °C) until assayed. Total metagenomic DNA was extracted from individual caecal samples using a QIamp DNA Stool Mini Kit (Qiagen, Hilden, Germany) after an additional bead-beating step. Bacterial composition was determined by sequencing of 16 s rRNA amplicons (V4-V5 region; 408 nt long) generated by a separate PCR reaction for each sample (in triplicate) using universal 16S primers, where the forward primer (5′-AYTGGGYDTAAAGNG), with attached molecular identifier tags between 454 adapter sequence and target-specific primer sequence, and the reverse primer V5 (5′-CCGTCAATTYYTTTRAGTTT) (Claesson et al., 2010), were used along with Biomix Red (Bioline, London UK). The template DNA was amplified under the following PCR conditions for a total of 35 cycles: 94 °C for 2 min and 1 min respectively (initialization and denaturation), 56 °C for 60 s (annealing) and 72 °C for 60 s (elongation), proceeded by a final elongation stage of 2 min. Negative control reactions with PCR grade water in place of template DNA were used to confirm a lack of contamination. Amplicons were pooled and cleaned using the AMPure XP purification system (Beckman and Coulter, Takeley, UK), and DNA concentration was determined using the NANODROP 3300 Fluorospectrometer (Thermo Scientific, USA) coupled with the Quant-it™ Picogreen® dsDNA Assay Kit (Invitrogen, Paisley, UK). Equal volumes of each sample were then pooled together and underwent a final cleaning and quantification stage. Amplicons were sequenced in-house on a Roche GS FLX Titanium platform.

2.5. RNA purification from mouse hippocampus and qPCR

Hippocampal mRNA expression in WT and APP transgenic mice was studied by quantitative PCR. The hippocampi were homogenized in TRIzol reagent (Invitrogen, Carlsbad, CA, USA), extracted, and the first-strand cDNA was synthesized from 2 μg RNA using M-MLV reverse transcriptase (Promega, Madison, WI, USA) according to the manufacturer's protocol.

Diluted cDNA was amplified by real-time PCR in a 15 μL volume reaction in a 7500 Real-Time PCR System (Applied Biosystems, Warrington, UK) with Power SYBR® Green reagent (Applied Biosystems). Gene expression was determined with 7500 Software v2.0.4 using ROX as a passive reference dye. A standard curve, with varying dilutions of each sample mix, was performed for each primer set to ensure the presence of unique amplification products. cDNA amplification was done by using conventional Applied Biosystems cycling parameters (40 cycles of changing temperatures, first at 95 °C for 15 s and then 60 °C for a minute).

Ct (cycle threshold) values for all the genes analyzed ranged between 12 and 31. Data were represented using the comparative Ct method, and for a valid DDCt value, we verified that the efficiency of amplification of the target and the reference gene is approximately equal (the absolute value of the slope of DCt vs. log relative concentration should be between −0.1 and 0.1). The Ct was determined for each target gene in duplicate. DCt was calculated by the difference between the Ct of each target gene and the Ct of an artificial BestKeeper reference gene based on the Ct values of 3 independent reference genes: RPL13A, RPS29, and β-actin calculated using the BestKeeper© Software (http://gene-quantification.com/bestkeeper.html). This approach helps to determine stable housekeeping genes, differentially regulated target genes and sample integrity (Pfaffl et al., 2004). We studied the mRNA expression levels of the set of genes listed in suppl. Table 2.

2.6. Bioinformatic and statistical analysis

Statistical significance analysis was assessed by using two-way or one-way ANOVA, followed by Bonferroni's post hoc test or by unpaired Student's *t*-test (Prism software, version 5.0; GraphPad Software, San Diego, CA). A value of $p < 0.05$ was considered significant.

16S microbiota analysis was as follows. Paired-end reads were assembled using FLASH (FLASH: fast length adjustment of short reads to improve genome assemblies). Further processing of paired-end reads, including quality filtering based on a quality score of >25 and removal of mismatched barcodes and sequences were completed using quantitative insights for microbial ecology (QIIME) version 1.9.0. Denoising, chimera detection, and clustering into operational taxonomic units (OTU) grouping were performed using USEARCH v7. OTUs were aligned using PyNAST, and taxonomy was assigned using BLAST against the SILVA SSURef database release 119. To assess the beta diversity in bacterial communities, unweighted and weighted UniFrac distances, a distance metric often used to separate groups in microbiome analysis, were used to perform principal co-ordinate analysis (PCoA). Statistical analysis was performed using the Calypso online software (version 8.68) and Prism (version 5.0). Cumulative-sum scaling was used and data were log2 transformed to account for the non-normal distribution of taxonomic count data for alpha and beta diversity testing. All the microbiota data was analyzed using the statistical software package SPSS 21.0 (IBM). The rank Kruskal-Wallis test was used for comparison of taxa (abundance >2%) followed by Dunn-test with Bonferroni *p*-value adjustment for multiple comparisons.

3. Results

3.1. Stress-like effects of HFD in WT and TgAPP mice in a sex-dependent manner

First, we studied motor activity in an open field for 10 min. No overt differences in locomotion and rearing were observed between male or female WT or TgAPP mice on the LFD (Fig. 1). However, male TgAPP spent more time in the margins compared to WT mice, while.

no difference was found in females LFD fed. Both horizontal activity, as judged by the distance traveled over a 10 min period, and rearings were diminished by the HFD regime (Fig. 1, A and B), with diet having an overall effect (suppl. Table 3). These reductions reached statistical significance in the case of male WT mice and female TgAPP on the HFD. The time spent in the margins of the arena was significantly decreased in female WT mice on the HFD, perhaps pointing to disinhibition. In contrast, it was increased by two-fold in male WT mice. Male TgAPP LFD mice spent more time in the margins in comparison with the WT LFD group, as already mentioned, and HFD did not modify this parameter (significant interaction between genotype and diet, Suppl. Table 3). Taken together, these results suggest that HFD induces anxiety in a sex-dependent manner, being more obvious in male mice.

However, male TgAPP spent more time in the margins compared to WT mice, while no difference was found in females LFD fed.

Using the TST we further explored the possible anxiety shown by the mice submitted to different diets (Fig. 2). HFD tended to increase the immobility time of male mice, both WT and TgAPP, and significantly enhanced that of WT female mice. In contrast, the immobility of HFD treated female TgAPP mice was reduced to approximately 60% of that of female WT mice on HFD (significant interaction between genotype and diet in females, Suppl. Table 3). The number of fecal pellets mirrored the TST results. In fact, HFD markedly increased the number of pellets of male WT and reduced that of female TgAPP mice. Finally, no correlation between the immobility time and weight was found (Suppl. Fig. 2).

All animals were submitted to the passive avoidance test to assess learning of aversive memory. We did not found any difference in the latencies to enter the dark compartment, where the electric shock was inflicted (suppl. Fig. 1). Therefore, aversive memory was preserved in mice of differing genotype, sex and diet consumption.

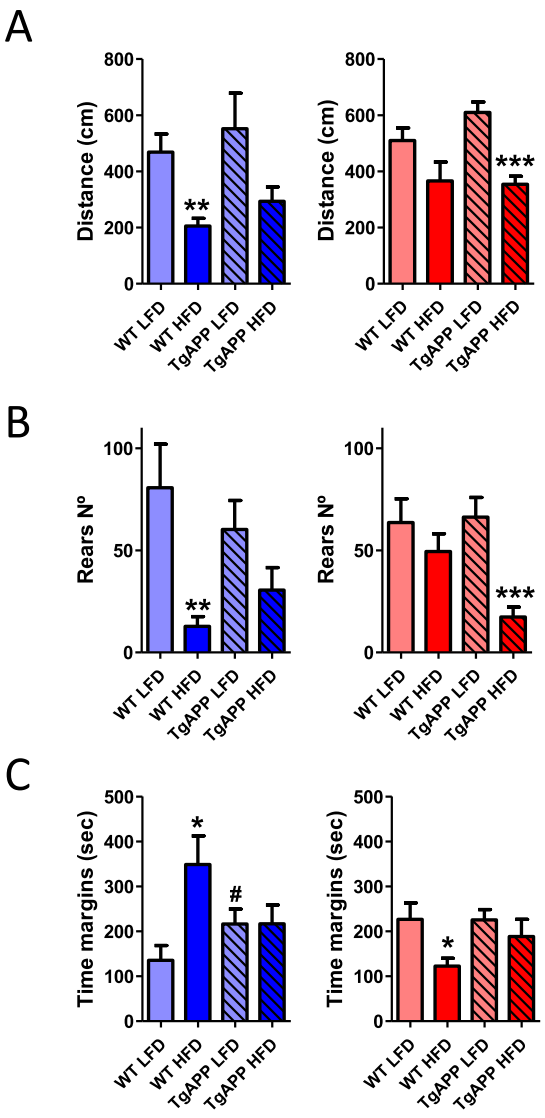


Fig. 1. HFD decreased motor activity in WT and TgAPP mice. Mice were fed HFD or LFD for 18 weeks, starting at 7 months of age. A. Distance traveled (cm) was decreased by HFD in female TgAPP and male WT mice compared to LFD treated mice. B. HFD reduced rears (n°) of female TgAPP and male WT mice compared to LFD treated mice. C. Male TgAPP on LFD mice spent more time in the margins vs WT LFD mice. The time spent in the margins was increased in male WT by HFD but was decreased in female WT mice. Bar graphs represent the mean ± SEM of 5 mice per experimental group for the WT mice and 9 mice per experimental group for the TgAPP mice HFD decreased time in margins of female WT LFD fed. Bar graphs (male: blue; female pink/red) represent the mean ± SEM of 5 mice per experimental group for the WT mice and 9 mice per experimental group for the TgAPP mice. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. LFD fed mice; #p < 0.05 vs. WT mice. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.2. Gut microbiota is influenced by HFD: effects of sex

It is well known that HFD feeding induces gut microbiota dysbiosis, including decreased diversity and microbiota composition changes. Thus, we performed a systematic study at the phylum, family, and genus level of gut microbiota from the caecal content.

The β-diversity of the ceacal microbiota community was assessed by PCoA analysis at the OTU level using weighted UniFrac (quantitative)

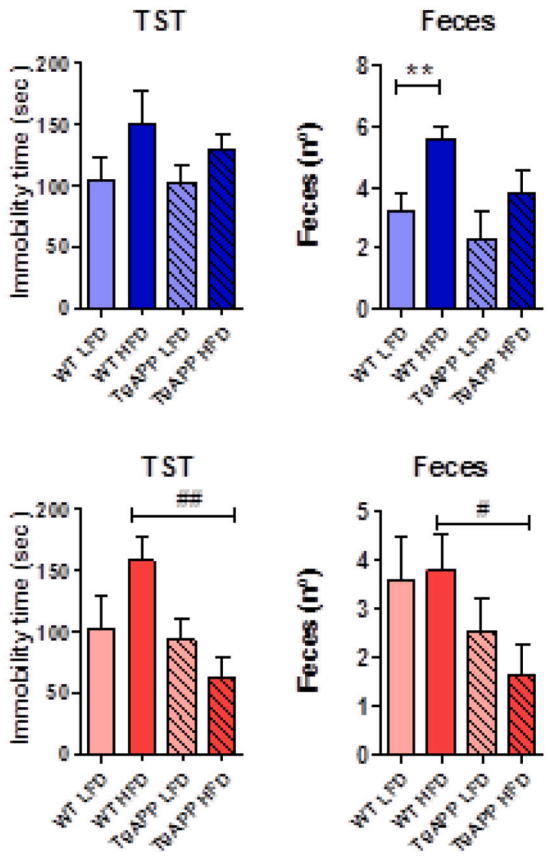


Fig. 2. HFD altered anxiety-related behavior in WT and TgAPP mice. Animals were submitted to a tail suspension test (TST) for 6 min, and immobility time (*sec*) was recorded. The number of fecal pellets (Feces) was counted while the animals were in the open field (10 min). HFD decreased immobility and fecal pellets of female TgAPP mice compared to WT HFD. In contrast, HFD increased the number of fecal pellets of male WT mice. Bar graphs (male: blue; female pink/red) represent the mean ± SEM of 5 mice per experimental group for the WT mice and 9 mice per experimental group for the TgAPP mice. **p < 0.01 vs. LFD fed mice, #p < 0.05 and ##p < 0.01 vs. WT mice. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and unweighted UniFrac (qualitative) distances (Fig. 3A). A significant clustering of the HFD groups from LFD groups was found using both weighted (Adonis $p < 0.001$, R2 = 0.158) and unweighted UniFrac (Adonis p < 0.001, R2 = 0.183). Sex differences were observed within the HFD groups using unweighted UniFrac, with significant clustering of male and female groups observed (Adonis p < 0.001, R2 = 0.266). Interestingly, the female TgAPP-LFD group clustered significantly from all the other groups except the WT-LFD female group based on weighted UniFrac (Fig. 3A). In summary, we observed a diet and sex effect on taxa qualitative abundance and a trend for taxa quantitative abundance in female TgAPP mice that received the LFD. Moreover, microbiota α-diversity was analyzed using Shannon and Simpson indexes (Fig. 3B). Overall, HFD decreased gut microbiota α-diversity in both male and female WT and TgAPP mice. This decrease was significant, except for TgAPP female mice when using Simpson index.

No major effects were observed in the most abundant phyla (*Firmicutes* and *Bacteroidetes*) across the groups (Fig. 4A and suppl. Table 4). However, despite their scarce abundance, we found a significant sex effect on *Verrucomicrobia* abundance in TgAPP-LFD female mice, which was markedly increased. Moreover, when analyzing male and female groups separately, HFD feeding effects were observed. Thus, HFD-fed male WT mice and female TgAPP mice showed significantly increased abundance of *Verrucomicrobia* and *Deferribacteres*, respectively. The *Firmicutes/Bacteriodetes* ratio, which has been generally considered an eventual obesity biomarker, was also analyzed (Fig. 4B). Indeed, HFD tended to increase this ratio in both male and female mice, independently of genotype, being significant in the case of male TgAPP mice (Fig. 4B).

Next, we examined caecal microbiota at the family level (Fig. 5 and suppl.Table 5). In TgAPP mice fed a LFD, we found a remarkable increase in *Verrumicrobiacea* in female but not in male mice. HFD significantly decreased *Porphyromonadaceae* abundance in both male and female TgAPP mice, and tended to diminish in WT of both sexes. *Deferribacteraceae* was also enhanced by HFD in TgAPP females. In the case of female mice, HFD led to significantly decreased abundance of *Coriobacteriaceae* in both WT and TgAPP mice. Moreover, when considering only female groups in the comparisons, significant HFD feeding effects were observed in TgAPP mice with decreased abundance of both *Prevotellaceae* and *Erysipelotrichaceae*, while *Helicobacteraceae* and *Deferribacteraceae abundance* increased. Sex effects were also found at this phylogenetic level. Thus, *Deferribacteraceae*, *Verrucomicrobiaceae*, and *Erysipelotrichaceae* showed higher abundance in female mice in WT-LFD, TgAPP-LFD, and TgAPP-HFD groups, respectively (Fig. 5 and suppl.Table 5).

The analysis at the genus level is shown in Fig. 6 and suppl.Table 6. Again female TgAPP mice submitted to a LFD showed significant differences. Thus, an unidentified uncultured genus from *Ruminococcaceae* was decreased while *Akkermansia* was strikingly increased in this group when compared to female WT mice (genotype effect) and TgAPP male (sex effect) mice fed a LFD respectively. HFD resulted in significant changes in the relative abundance of different genus. The only changes induced by HFD in WT mice were an increase in *Akkermansia* in male and a decrease in both the abundance of an unidentified genus from the *Bacteroidales* family and an unidentified genus in female mice. Moreover, in female TgAPP mice, HFD intake increased an unidentified genus from the *Ruminococcaceae* family, *Helicobacter* and *Mucispirillum*, and it decreased *Alistipes*, an unidentified genus from the *Bacteroidales* family, *Allobaculum* and another unidentified genus from *Coriobacteriaceae* (also reduced in WT females by HFD). In contrast, the reduction in *Parabacteroides* abundance by HFD occurred in both male and female TgAPP mice. As shown in suppl.Table 6, statistical analysis found sex effects. Thus, females showed a significant decrease in the relative abundance of the unidentified genus belonging to the *Bacteroidales* family in WT-HFD groups and *Alistipes* in TgAPP-HFD groups, as well as a significant increase in *Akkermansia* and *Mucispirillum* in WT-LFD and TgAPP-HFD groups respectively, in comparison with their male counterparts. Finally, a significant decrease in the relative abundance of an uncultured genus belonging to the *Ruminococcaceae* family was observed in TgAPP LFD female mice when comparing only the female mice groups.

In summary, we found that female TgAPP was characterized by a huge increase in the genus *Akkermansia* (phylum *Verrumicrobia* and family *Verrumicrobiacea* significantly enhanced as well) when comparing to TgAPP male mice, and decreased *Ruminococcaceae* uncultured genus when comparing to female WT mice. Interestingly, HFD significantly altered a few gut microbiota in male WT and TgAPP mice. In contrast, TgAPP females showed many changes in gut microbiota abundance at all taxonomic levels. Mice tend to perform coprophagy. Therefore, in our housing conditions, the microbiota changes observed in TgAPP mice would be lower in comparison with singly housed mice.

3.3. HFD modifies the expression of neuroinflammatory genes in the mouse hippocampus

Next, we assessed the mRNA expression of several pro-inflammatory mediators, and the results are shown in Fig. 7. Cluster of differentiation 68 (CD 68) was the only gene with no significant differences between the study groups.

ANOVA (suppl. Table 7) revealed a significant diet effect on interleukin-6 (IL-6) mRNA levels in male mice, an overall effect of

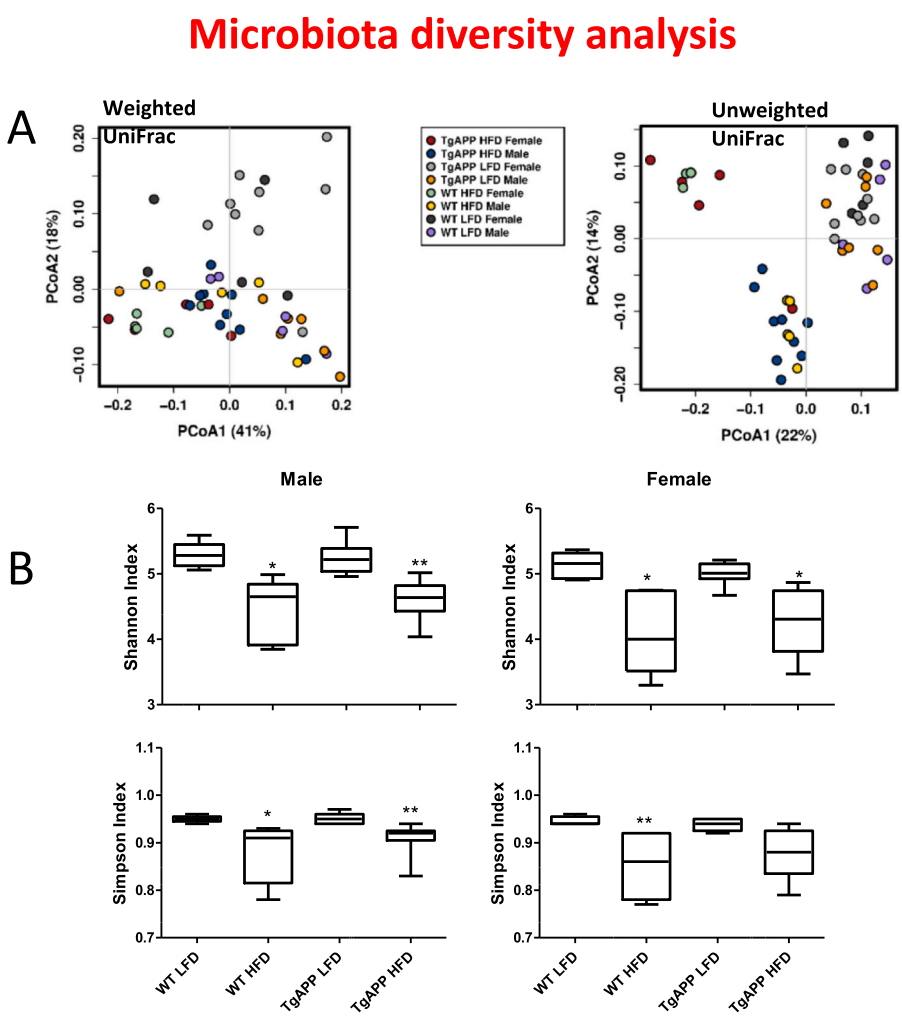


Fig. 3. Effect of HFD on microbiota diversity in caecal samples from wild type (WT) and TgAPP mice. (A) Principal coordinate analysis (PCoA) based on weighted and unweighted UniFrac distances. The percentage of variation explained is indicated on the respective axes. (B) Alpha diversity calculated by Shannon and Simpson indexes. $*p < 0.05$; $**p < 0.01$ indicates significant high-fat diet (HFD) effect analyzed by Kruskal-Wallis followed by Bonferroni correction for multiple comparisons).

genotype, and a genotype by diet interaction in mice of both sexes. In males, there was an overall increase in IL-6 m RNA levels in TgAPP mice, with a huge increase (8 fold) in male TgAPP mice on the HFD. HFD had no effect in WT male mice. In female mice, IL-6 expression was differently regulated. Indeed, female TgAPP fed a LFD showed a marked increase in IL-6 expression levels compared to WT-LFD mice. In WT females, HFD enhanced IL-6 expression, but in female TgAPP mice HFD decreased IL-6 mRNA levels compared to TgAPP females on a LFD. IFNγ mRNA levels were increased to a similar extent in both female and male TgAPP mice, and HFD did not modify these levels in either WT or TgAPP mice.

IL-12β expression levels were not significantly affected by diet or phenotype in male mice (Fig. 7 and suppl. Table 7). In contrast, HFD markedly increased IL-12β levels in WT females, but had no effect in TgAPP females. However, on a LFD female TgAPP mice had increased IL-12β mRNA levels compared to WT mice. The expression of IL-1β was increased 4 fold by HFD in male WT mice, and the 2 fold increase in male TgAPP LFD mice compared to WT on LFD was not modified by the HFD. In the case of the female groups, we found no statistically significant changes in IL-1β mRNA levels, although there was a trend towards higher levels in female TgAPP mice. In male mice, the expression levels of TNF-α mRNA were not altered by diet or genotype. However, a remarkable increase (8 fold) in TNF-α expression was observed in female TgAPP mice versus WT mice that received LFD, with this increase being completely absent in TgAPP on HFD.

In summary, we found that pro-inflammatory markers did not differ overall between male and female WT mice, and that in TgAPP mice the expression of some cytokines was increased compared to WT mice, in particular in females. On the other hand, HFD in male TgAPP did not alter mRNA levels of pro-inflammatory markers compared to LFD fed transgenic mice, with the exception of IL-6, which was specifically increased. On the contrary, in female TgAPP mice there was an HFD reduction of the genotype-associated increase in inflammatory markers.

In Fig. 8 the mRNA levels for different genes involved in phagocytic responses are depicted. Few differences in mRNA expression were observed due to sex (suppl. Table 7): CD36 expression was lower, while MHCII and mannose receptor mRNA levels were higher in female WT (LFD fed) mice compared to male WT mice. HFD decreased CD36 mRNA expression levels in male WT mice, but it did not alter the levels in male TgAPP mice. In female WT mice, HFD had no effect, while HFD markedly reduced the huge increase in CD36 expression in female TgAPP. Both male and female WT mice on HFD showed a marked increase in MHCII expression. While HFD did not modify mRNA levels of MHCII in male TgAPP mice, the reduced levels found in female TgAPP mice were normalized by HFD. The expression of interleukin-1 receptor-associated kinase 4 (IRAK 4) was significantly diminished by HFD treatment in male TgAPP mice with no other differences found in males. However,

HFD abrogated the marked increase in IRAK mRNA levels in female TgAPP mice. In male mice, there were no differences between groups in P2RY6 expression. In females, HFD did not affect P2RY6 mRNA levels in WT mice, but reversed the marked increase observed due to the APP genotype. HFD did not alter the expression of mannose receptor in male WT mice; however, it normalized the levels that were increased (2 fold) in male TgAPP receiving LFD, while no effects of diet or genotype were

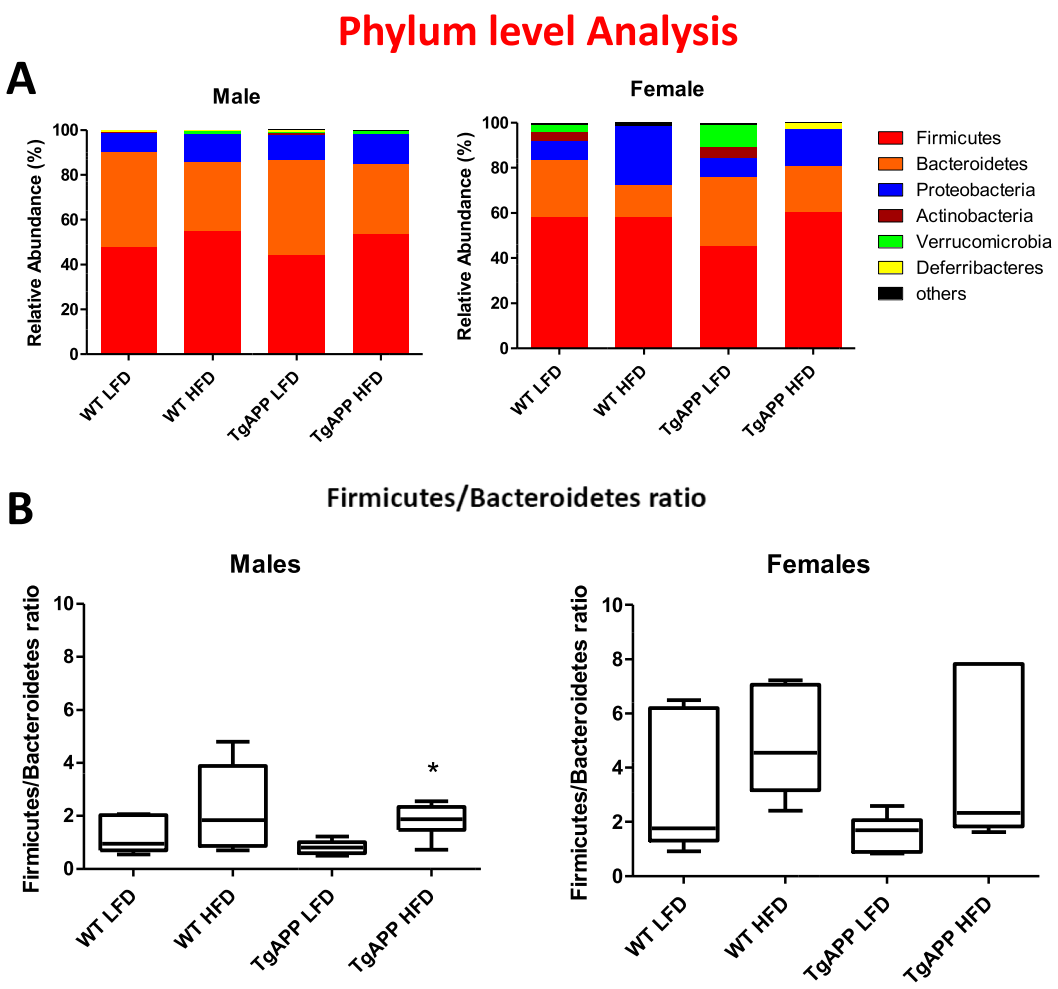


Fig. 4. Effect of HFD on caecal microbiota composition at the phylum level from wild type (WT) and TgAPP mice. (A) Stacked bar plots showing the average relative abundance of different bacteria phyla (B) Box-and-Whisker plot of *Firmicutes* to *Bacteroidetes* ratio. Boxes represent the interquartile range (IQR) between the first and third quartiles (25th and 75th percentiles, respectively), and the horizontal line inside the box defines the median. Whiskers represent the lowest and highest values. *p < 0.05 indicates significant HFD effect in male TgAPP mice (Kruskal Wallis followed by Bonferroni correction for multiple comparisons).

observed in female mice. In male mice there were no effects of either diet or genotype on TREM2 mRNA levels. In contrast TREM2 mRNA levels were decreased in female TgAPP mice compared to female WT and HFD normalized these levels, while having no effect in WT females.

Thus, we had found little if any regulation of genes involved in phagocytosis in male mice due to diet or genotype; however, in female mice genes that were either increased (CD36, IRAK4 and P2RY6) or decreased (MHCII and TREM2) in TgAPP returned to control levels (those of WT on LFD) when on HFD. Only in the case of MHCII was there an effect of HFD in WT mice with this diet inducing a 3 fold increase but only in females.

4. Discussion

In this study we found that WT mice fed HFD displayed alterations in anxiety-like behavior, increased hippocampal pro-inflammatory cytokine expression, and marked changes in microbiota composition, when compared with LFD-fed mice. As expected, the effects of the diet were different between sexes. In the context of AD, prolonged HFD induced few effects on mRNA expression of inflammatory/phagocytosis-related genes in male mice. However, female TgAPP mice on an HFD displayed an altered anxiety-like response, the genotype-induced increase in gene expression returned towards control levels, while microbiota showed significant changes at all taxonomic levels induced by HFD feeding.

Several reports have shown that HFD induces an anxiogenic profile (André et al., 2014; Eidson et al., 2019; Gainey et al., 2016). In addition, in AD patients, increased anxiety and/or depression are reported to occur over the course of the illness (Ferretti et al., 2001; Lyketsos et al., 2002). However, given that most studies have been performed on male animals, possible sex differences have gone unnoticed. There were no evident differences in motor activity in the open field test between male and female mice, whether WT or TgAPP, in accordance with previous reports (Hwang et al., 2010; Lee et al., 2006), with the exception of an increase in time in the margins in male TgAPP mice. In contrast, HFD appeared to induce an anxiety-like phenotype, as judged by the marked decrease in locomotion and rearing, the increased time in the open field margins, and the increased number of fecal pellets in male WT mice. Indeed, other studies have found a decrease in both locomotion and time spent in the center in the open field following prolonged HFD in male mice (Almeida-Suhett et al., 2017; Gainey et al., 2016). In the case of female mice on an HFD, overweight may impact on the open field parameters, since we observed an inverse correlation with weight, absent in the case of males (suppl. Fig. 2). Even though both WT and TgAPP showed a similar weight gain, in female WT mice HFD non-significantly diminished locomotion and rearing, while it reached significance in female TgAPP. In contrast to male WT mice, HFD in females decreased the time spent in the margins indicating decreased anxiety-like phenotype or disinhibition, but again overweight may be a confounding factor. Therefore, HFD affected anxiety-like behavior differently depending on

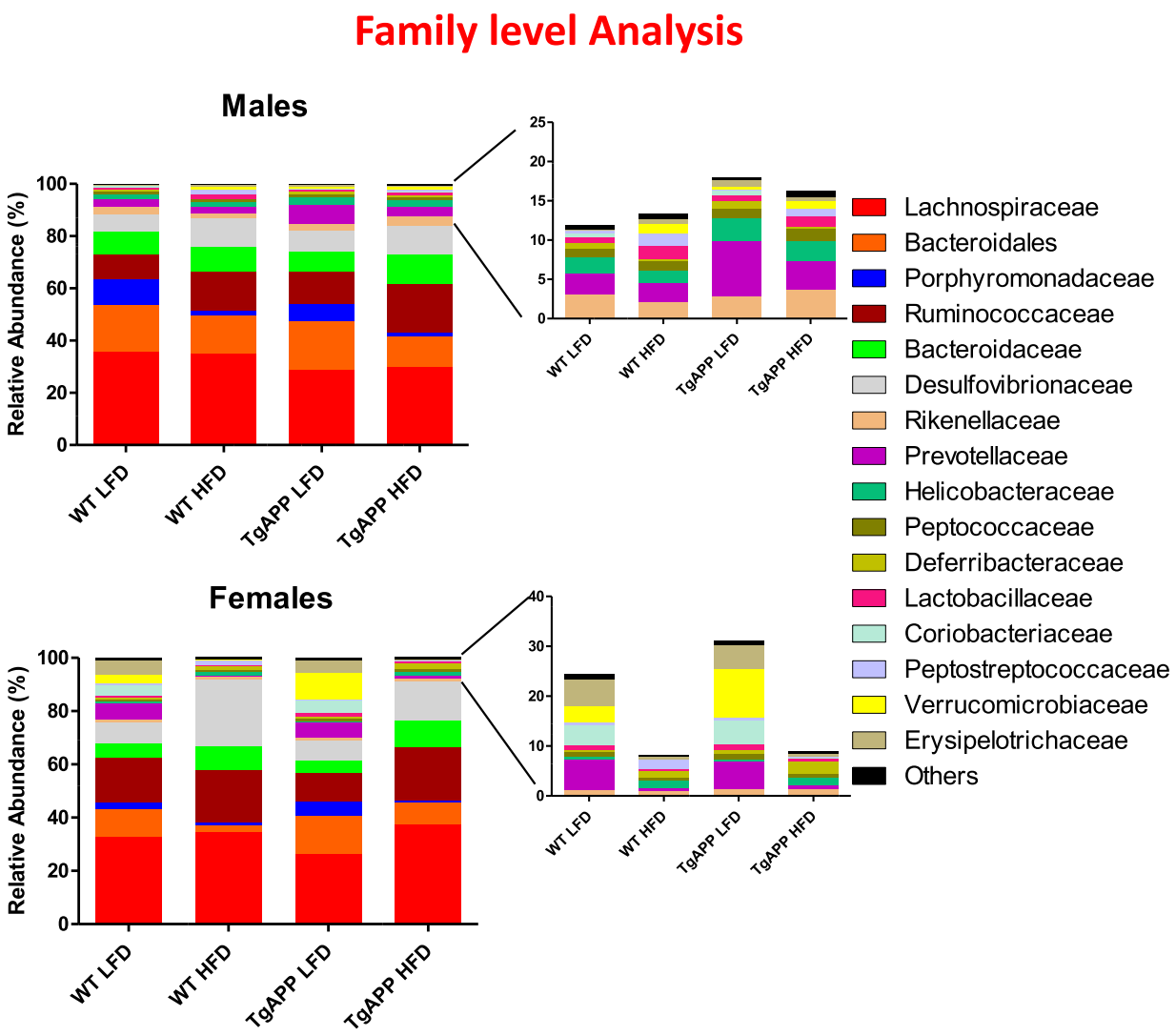


Fig. 5. Effect of HFD on caecal microbiota composition at the family level of wild type (WT) and TgAPP mice. Stacked bar plots showing the average relative abundance of different bacteria families. For the sake of clarity low abundance families have been enhanced apart.

the sex. In agreement with our results, Bridgewater et al. (2017) found increased anxiety-related behaviors in male mice on an HFD while females showed no such changes.

Several studies have reported that HFD induces depressive-like symptoms, with mice on an HFD showing increased immobility in the forced swim test and reduced exploratory behavior (Sharma and Fulton, 2013; Almeida-Suhett et al., 2017). Moreover, HFD fed mice are reported to display anhedonia, increased plasma corticosterone levels, under basal conditions or following stress exposure, and alterations in the forced swim test, all indicative of anxiety and/or depressive-like behavior (Agusti et al., 2018). In the present study, we selected the TST, instead of FST, since we considered that it was better suited for overweight mice. We anticipated that animals on an HFD will become obese, and increased weight would impact their swimming abilities. Moreover, the TST avoids hypothermic exposure compared to FST (Cryan et al., 2005). Finally, although TST depends on a motor readout, mice showing reduced locomotion in the OFT would not be necessarily impaired in the TST. An example is the reduced immobility in the TST of mice after acute drug administration, which may suppress activity in the OFT (as is the case of many antidepressants) (Cryan et al., 2005). In the TST, we found that HFD induced a tendency towards increased anxiety-like behavior, as judged by the enhancement in immobility time, in both male and female WT mice, but this was not observed in TgAPP mice. Indeed, female TgAPP mice on an HFD displayed decreased anxiety-like behavior since they showed decreased immobility time in the TST and fecal pellet production compared to WT mice on the same diet (HFD). This result was unexpected given their enhanced body weight, although immobility time was not significantly correlated with weight in either male or female mice (suppl. Fig. 3). Hence, female TgAPP on the HFD appeared to be resilient to the stressful conditions in the TST.

Analysis of microbiota revealed several interesting findings. Weighted and unweighted measures of β-diversity showed marked diet and sex, but little genotype effects influencing the microbial communities in male and female groups. Regarding the qualitative taxa abundance, groups mostly clustered according to the type of diet, in agreement with other authors (Bridgewater et al., 2017; Kong et al., 2019; Shi et al., 2020). Indeed, diet is one of the main factors shaping the gut microbiota composition and this has been extensively reported in many studies (e.g. Cani and Everard, 2016; Wu et al., 2011). Moreover, in the case of HFD groups, they are grouped depending on the sex. This is in accordance with other studies where differences in gut microbiota composition have been observed for HFD-fed animals of different sex (Bridgewater et al., 2017; Shi et al., 2020). These changes in β diversity rely on diet and sex effects on the relative abundance of several bacteria taxa at different phylogenetic levels including phylum, family and genus levels. Interestingly, based on quantitative taxa abundance, a TgAPP

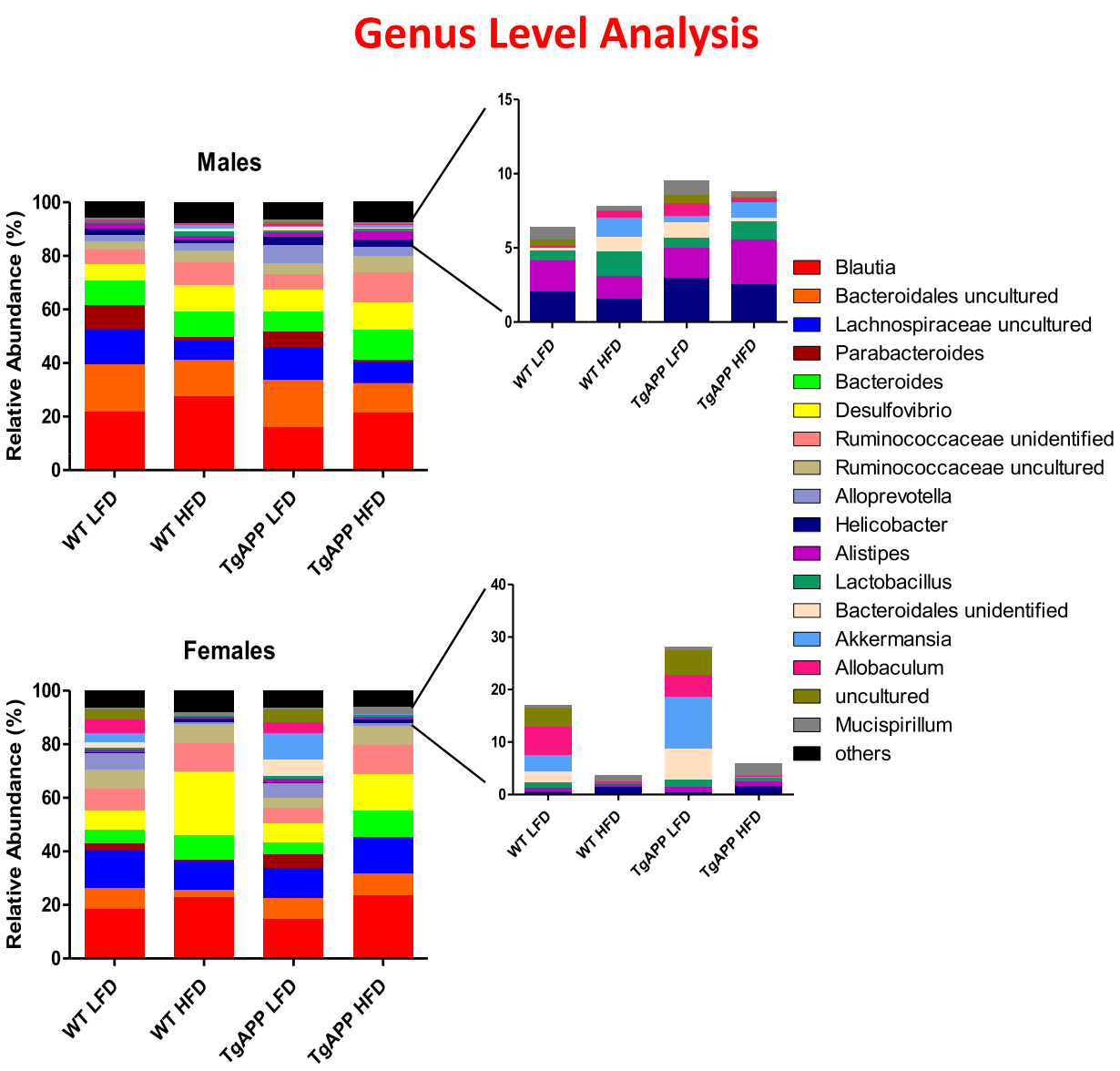


Fig. 6. Effect of HFD on caecal microbiota composition at the genus level of wild type (WT) and TgAPP mice. Stacked bar plots showing the average relative abundance of different bacteria genera. For the sake of clarity low abundance families have been enhanced apart.

effect was observed in the case of LFD-fed female mice, as this group clustered differently from all other groups, and this may be related to the greater susceptibility of females to AD-induced changes (see Introduction). Notably, ceacal microbial communities separated between control and AD patients at all taxonomic levels as well (Zhuang et al., 2018). In female TgAPP mice we observed a significant decrease in an uncultured genus belonging to the *Ruminococcaceae* family, in agreement with a very recent report (Cuervo-Zanatta et al., 2021). *Ruminococcaceae* has recently been shown to be markedly reduced in AD patients, an alteration that distinguished gut microbiota in Alzheimer's disease from that observed in amnestic mild cognitive impairment in a Chinese cohort (Liu et al., 2019). However, in that study they also reported decreased *Firmicutes* and increased *Proteobacteria*, and other authors have also shown a decrease in *Firmicutes*, with an increase in *Bacteriodetes* abundance in AD transgenic mice (Bäuerl et al., 2018; Cox et al., 2019; Harach et al., 2017), but these changes were not observed here.

We found a striking increase in *Akkermansia* in female TgAPP on LFD (phylum *Verrumicrobia* and family *Verrumicrobiacea* significantly enhanced as well). The abundance of this genus is inversely correlated with body weight and type 1 diabetes, both in humans and mice (Karlsson et al., 2012; Hansen et al., 2012). Moreover, *Akkermansia* decreased in genetically obese mice and type 2 diabetes, following HFD feeding, and treatment with that bacteria improved diet-induced effects (Everard et al., 2013). These findings suggest that increased *Akkermansia* abundance in female TgAPP may confer some advantage against the effects of HFD, but we found that the marked enhancement was lost in female TgAPP submitted to HFD. Interestingly, it has recently been shown that treatment with *Akkermansia* could delay the brain pathological changes and improve impaired cognition and anxiety-related behaviors in a mice model of AD (Ou et al., 2020). This is in accordance with our open field results. Thus, male TgAPP mice fed a LFD showed increased time in the margins compared to their respective WT control, indicating increased anxiety like behavior, while this was not observed in female TgAPP mice.

As expected, HFD reduced both microbiome richness and diversity (Shi et al., 2020; Kong et al., 2019). Few changes were observed in WT mice of both sexes following HFD consumption. Thus, we found increased *Verrumicrobia* and *Akkermansia* in male WT mice on an HFD, also reported by other authors (Shi et al., 2020). We did not observe significant changes due to sex in WT mice in the present work, although

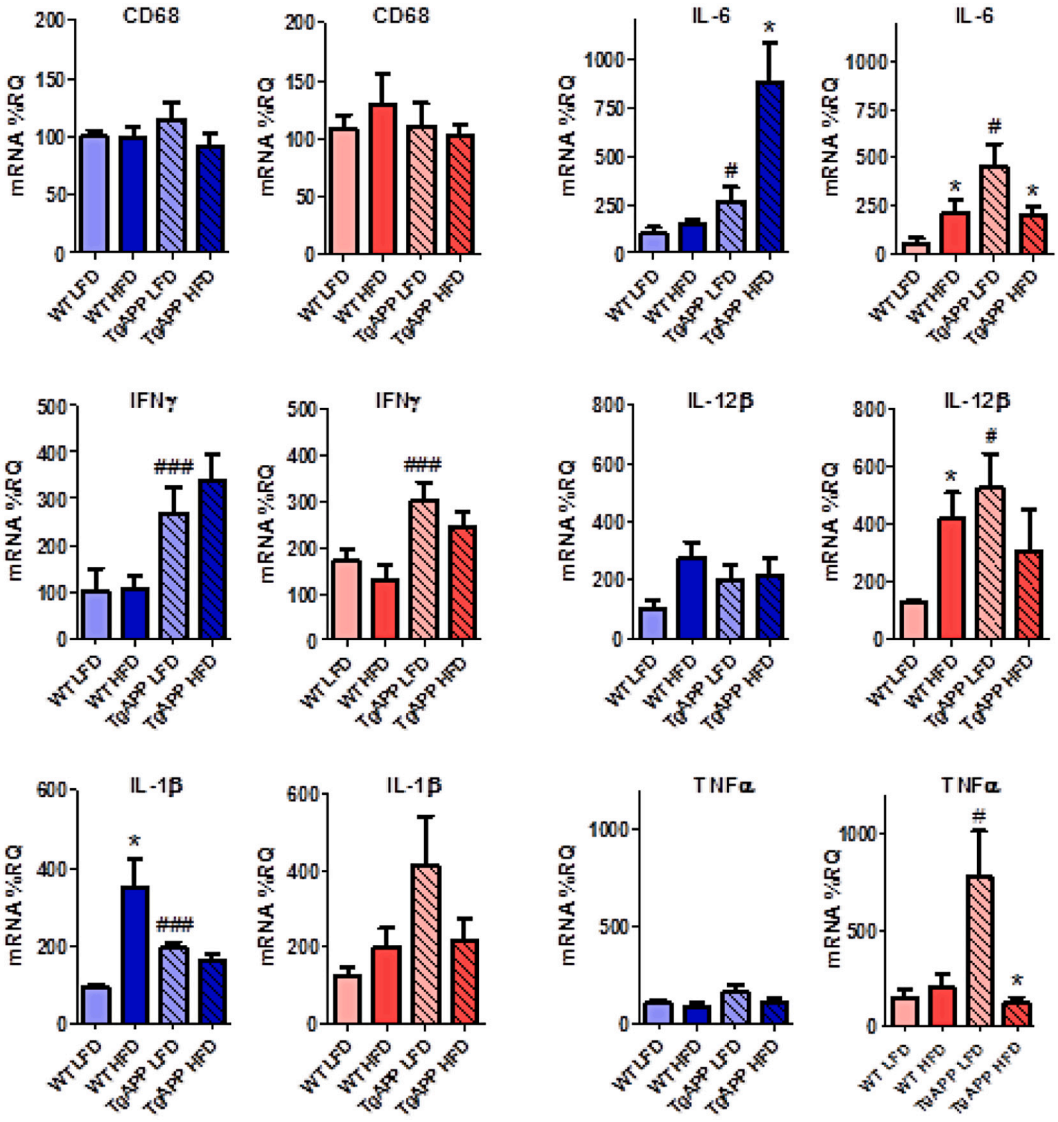


Fig. 7. A high-fat diet (HFD) increases the mRNA expression of pro-inflammatory genes in the hippocampus of wild type and TgAPP mice. mRNA expression (measured as %RQ) of inflammatory genes Cluster of Differentiation 68: CD68; interleukin 6: IL-6; Interferon-gamma: IFNγ; Interleukin-1β, IL-1β; Interleukin-12β, IL-12β; Tumor necrosis factor α, TNFα) in the hippocampus of WT mice and TgAPP mice. Bar graphs (male: blue; female pink/red) represent the mean ± SEM of 5 mice per experimental group for the WT mice and 9 mice per experimental group for the TgAPP mice. * $p < 0.05$ compared to LFD; # $p < 0.05$ and ### $p < 0.001$ compared to WT. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

there are reports in the literature (Bridgewater et al., 2017; Cox et al., 2019; Jaggar et al., 2020; Shi et al., 2020), likely due to the small number of WT mice per group. However, we describe marked differences induced by HFD on the microbiota of TgAPP mice of different sex. Indeed, female TgAPP mice showed significant changes at all taxonomic levels induced by HFD feeding. These changes were more evident at the genus levels, with significant decreases, for example in *Alistipes* (Kong et al., 2019), *Parabacteroides* and *Allobacolum*, with a marked enhancement of *Muscipirillum* (Kong et al., 2019), *Helicobacter* and a *Ruminoccaceae* unidentified. It is worth mentioning that, although not significant, HFD feeding increased *Proteobacteria* relative abundance in female WT mice, but this did not happen in female TgAPP mice, and it was neither observed in male mice groups. Bacteria from this phylum has been extensively linked to inflammation as they are able to utilize inflammatory by-products for their survival (Winter and Bäumler, 2014). Therefore, during chronic inflammation, such as that linked to HFD-induced obesity, there is an increase in their relative abundance due to their advantage over other intestinal bacteria that lack this metabolic capacity (Winter and Bäumler, 2014). Hence, the lower levels in *Proteobacteria* observed in TgAPP HFD females may be linked to the reduced expression of hippocampal inflammatory mediators also found in this group (see below), and may also explain their reduced anxiety levels.

The effects of microbiota alterations are mediated by several molecules produced by the gut bacteria, including amino acids, vitamins, small chain fatty acids (SCFAs) and neurotransmitters. These molecules have functional consequences, whether beneficial or detrimental. Recent works have described changes SCFAs in AD models, showing increased serum levels of butyrate, 3-Methyvalerate, and caproate in an AD rat model (Guo et al., 2020), while in Aβ injected mice lower levels of acetate and propionate were found in feces and hippocampus (Xu et al., 2020). Accordingly, in AD transgenic mice, acetate was decreased and propionate was increased in comparison with their WT counterparts, while butyrate concentrations were higher in WT females in comparison with the other groups of mice (Cuervo-Zanatta et al., 2021), and altered fecal SCFAs were found to correlate with recognition memory and anxiety levels (Cuervo-Zanatta et al., 2021). Other work showed increased serum levels of acetate and propionate in transgenic mice, and

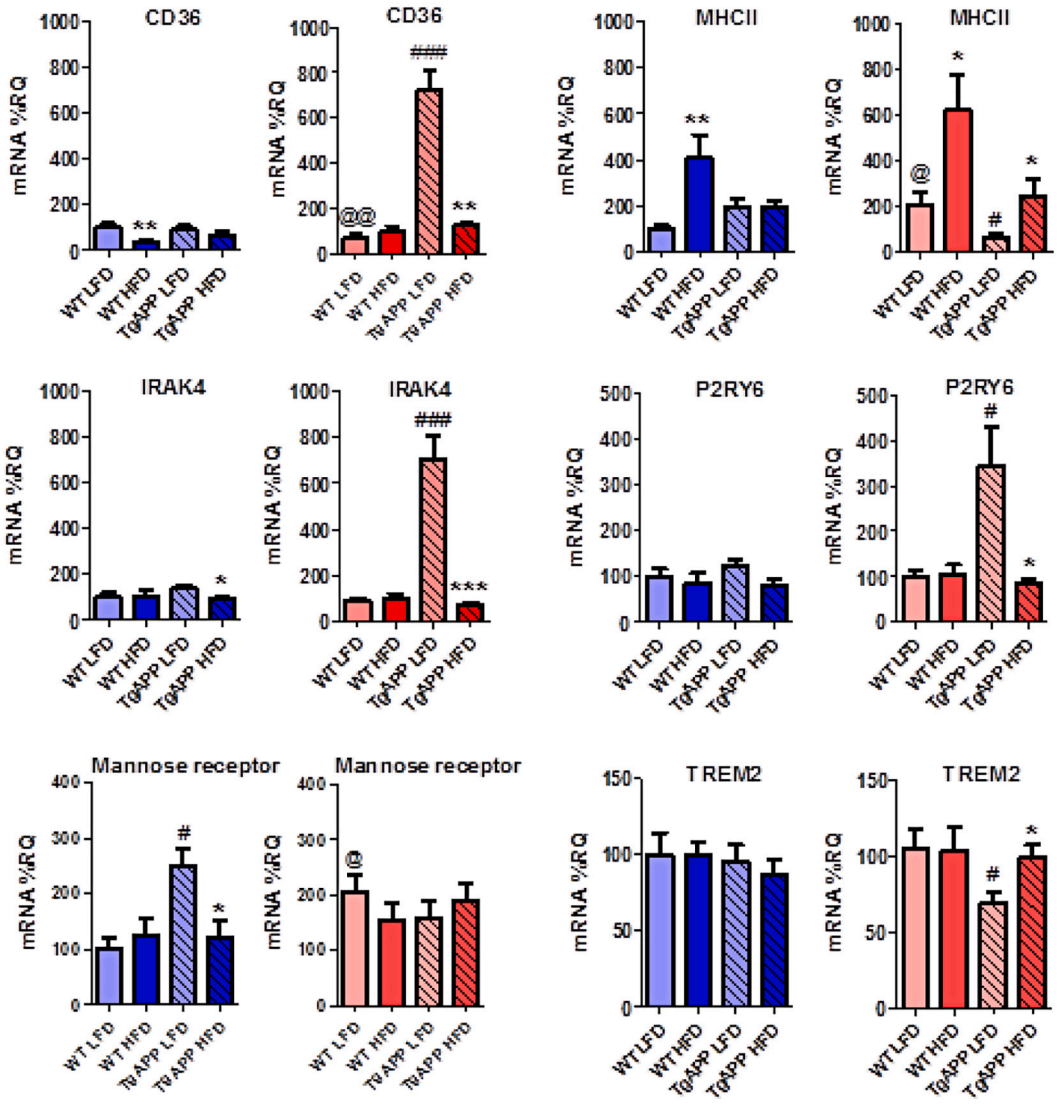


Fig. 8. High fat diet (HFD) alters the phagocytosis-related genes mRNA expression in the hippocampus of TgAPP mice. Relative mRNA expression (measured as %RQ) of phagocytosis related genes including Cluster of differentiation 36: CD36; Interleukin-1 receptor-associated kinase 4: IRAK4; Mannose receptor; Major histocompatibility complex class II: MHCII; Pyrimidinergic Receptor P2Y6: P2RY6; Triggering receptor expressed on myeloid cells 2: TREM2 in the hippocampus of WT and TgAPP. Bar graphs (male: blue; female pink/red) represent the mean ± SEM of 5 mice per experimental group for the WT mice and 9 mice per experimental group for the TgAPP mice. * $p < 0.05$ and *** $p < 0.001$ compared to WT; @ $p < 0.05$ and @@ $p < 0.01$ compared to LFD; @ $p < 0.05$ and @@ $p < 0.01$ compared to the other sex. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

no changes in butyrate or lactate (Kaur et al., 2020). Indoles produced by the gut microbiota are also emerging as mediators in the gut-brain axis and their possible interest for the treatment of AD has been recently reviewed (Pappolla et al., 2021). In fact, these molecules possess essential biological activities, including neuroprotective, anti-inflammatory, immunoregulatory, antioxidant and amyloid-β anti-aggregation properties. However, evidence of altered IPA levels or its possible effects in AD or models of the disease is lacking.

In accordance with previous reports showing increased hippocampal markers of inflammation in HFD-induced obese mice (Jayaraman et al., 2014; Kaur and Kaur, 2017; Tucsek et al., 2014), we found that HFD increases the pro-inflammatory profile in the hippocampus of male and female WT mice, with an increase in the expression of IL-1β, IL-6, IL-12β, and MHCII. These changes were more prominent in female WT mice on HFD, except for IL-1β expression, which was higher in male mice. In the context of AD, there was an increase in hippocampal IFNγ and IL-1β in both male and female TgAPP transgenic mice, as previously reported (Benzing et al., 1999; Patel et al., 2005). However, there was a remarkable enhancement of TNF-α, IL-12β and IL-6 mRNA levels in female TgAPP mice. This higher inflammatory environment in the female hippocampus could contribute to the higher occurrence of AD in women, particularly in the most elderly (Mazure and Swendsen, 2016). HFD had little effect on the inflammatory profile in male TgAPP mice hippocampi, except for IL-6 expression which was further increased compared to those mice on LFD. However, in TgAPP females HFD reduced pro-inflammatory gene expression towards WT control levels, a result that was unexpected. Beneficial as this decreased inflammatory environment might seem, it might be an indicator of the unresponsiveness of inflammation-related cell types, such as microglia or astrocytes. Thus, we analyzed different genes involved in inflammation-induced phagocytosis, such as CD36, IRAK4, MHCII, and P2RY6 (Fu et al., 2014; Hickman et al., 2008; Husemann et al., 2002; Xu et al., 2015). These genes are mainly expressed in microglia, the brain resident macrophage, although some are also expressed in other cell types. In WT mice of either sex, HFD was only found to decrease CD36 mRNA in male mice. In contrast, in the hippocampus of female TgAPP mice HFD

ablated the enhanced expression of CD36, IRAK4 and P2RY6, while in male TgAPP mice it reduced that of mannose receptor. Of note is the decreased TREM2 expression in female TgAPP, which resembles the different loss of function mutations in TREM2 found in the AD brain (Cuyvers et al., 2014; Colonna and Wang, 2016), and that has been shown to be affected by a western diet regime (Graham et al., 2016). Again, the HFD-induced changes in the phagocytosis-related genes were more prominent in the hippocampus of female TgAPP mice than in male TgAPP mice. These changes suggest that HFD impairs the phagocytic response of microglia in the TgAPP brain, and considering the importance of phagocytosis in maintaining brain homeostasis, it could be detrimental to the disease.

Several previous works have shown that HFD increases Aβ deposition and/or levels in different transgenic AD models (see Introduction), including Tg2576 mice used in the current study (Kohjima et al., 2010), although others reported no changes (Elhaik Goldman et al., 2018; Knight et al., 2014). Aβ has a crucial role in neuroinflammation and the increased pro-inflammatory cytokines observed both in clinical and experimental AD. As already mentioned, most works used mice of one sex, and more recently groups mixing both sexes. Barron et al. (2013) found that males and females transgenic mice studied separately showed similar higher hippocampal Aβ accumulation following HFD feeding. Therefore increased neuropathology after HFD could contribute to the inflammatory hippocampal environment observed in TgAPP mice, particularly in males. In our opinion, the blunted inflammatory responses in female TgAPP are difficult to reconcile with a further enhancement in Aβ due to the HFD, and make us suggest the involvement of differentially altered microbiota.

During the last decades, the inflammatory hypothesis of depression has gained momentum (Maes et al., 2009), since inflammatory markers are increased in depressed patients (Dowlati et al., 2010) and models of the disease (Yirmiya et al., 2001), the high comorbidity of depression with inflammatory disorders, and that most of the antidepressant drugs show anti-inflammatory activity (Liu et al., 2011; Yirmiya et al., 2001). The behavioral changes that we observed in mice following an HFD could be a consequence of the inflammatory response in the brain, as found in the hippocampus, but also in the periphery, as reported in another article with the same animals (in preparation). Indeed, we found enhanced expression of pro-inflammatory cytokines in the hypothalamus and in adipose tissue after continuous HFD feeding. On the other hand, HFD induced marked changes in the microbiota composition, likely producing gut leakage with concomitant endotoxemia. Therefore, the resilience of TgAPP females to the anxiety-inducing effects of HFD appears to be a combination of changes in microbiota, towards lower inflammatory taxons and the reduction of inflammatory markers in the hippocampus.

The major limitation of the current study is that the number of animals was not as high or as uniform as intended. Transgenic mice and their WT littermates were used and in the current cohort of animals, the transgenic mice outnumbered the WT mice, but that was beyond our control. In contrast, the strength of this study is the simultaneous comparison of the effects of diet, on behavior, fecal microbiota composition, at different taxonomic levels, and hippocampal inflammatory/phagocytosis-related gene expression in animals of both sexes and both genotypes. Indeed, previous studies report microbiota changes in different models of AD mice, as well as the effects of HFD on pro-inflammatory parameters in the brain, but few if any, have addressed the issue of sex. Undoubtedly one of the most striking results of our study is that female TgAPP mice appear to be resilient to the effects of HFD. They did not develop an anxiogenic phenotype, showed reduced expression of inflammatory mediators, and displayed differential microbiota composition.

AD is a multifactorial disease with reciprocal interactions between age, sex, genetic background, and environment (Sala Frigerio et al., 2019), which modulate the glial response to Aβ. Indeed lifestyle has strong effects on the onset and progression of the disease. Caloric restriction, exercise, and social engagement are highly beneficial, while HFD, inactivity, and social isolation have detrimental consequences in AD.

Declarations of Competing Interest

None.

Author contributions

Conceptualization: MLC, JAC, MAA, LMGS, NY-C, JFC; Investigation: MLC, NY-C, CTF, AC-C, SDP, CS, KH; Writing - Original Draft, MLC, JAC, MAA, LMGS, NY-C, CTF; Funding acquisition: MLC, JAC, MAA, LMGS, JFC.

Credit author statment

Author contributions: Conceptualization: MLC, JAC, MAA, LMGS, NY-C, JFC; Investigation: MLC, NY-C, CTF, AC-C, SDP, CS, KH; Writing - Original Draft, MLC, JAC, MAA, LMGS, NY-C, CTF; Funding acquisition: MLC, JAC, MAA, LMGS, JFC.

Acknowledgements

We thank Ms. Elisa Baides, Alba Garcimartín, Clara González-Martínez for skillful technical assistance. This work was supported by Ministerio de Economía, Industria y Competitividad (MINECO), Grant Numbers BFU2014-51836-C2-1-R to LMGS and MAA, BFU2014-51836-C2-2-R and BFU2017-82565-C21-R2 to JAC; Madrid Council S2010/BMD-2349 to MLC; Centre for Biomedical Network Research for Physiopathology of Obesity and Nutrition (CIBEROBN) to JAC, Centre for Biomedical Network Research for Frailty and Healthy Ageing (CIBERFES) to LMGS and MAA, and Centre for Biomedical Network Research for Neurodegenerative Diseases (CIBERNED) to MLC. AC-C was granted with a FPI fellowship by the MINECO (BES-2015-072980).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nbd.2021.105495.

References

Agusti, A., Moya-Pérez, A., Campillo, I., Montserrat-de la Paz, S., Cerrudo, V., Perez-Villalba, A., Sanz, Y., 2018. *Bifidobacterium pseudocatenulatum* CECT 7765 ameliorates neuroendocrine alterations associated with an exaggerated stress response and Anhedonia in obese mice. Mol. Neurobiol. 55, 5337–5352. https://doi.org/10.1007/s12035-017-0768-z.

Akiyama, H., Barger, S., Barnum, S., Bradt, B., Bauer, J., Cole, G.M., et al., 2000. Inflammation and Alzheimer's disease. Neurobiol. Aging 21, 383–421. https://doi.org/10.1016/s0197-4580(00)00124-x.

Almeida-Suhett, C.P., Graham, A., Chen, Y., Deuster, P., 2017. Behavioral changes in male mice fed a high-fat diet are associated with IL-1β expression in specific brain regions. Physiol. Behav. 169, 130–140. https://doi.org/10.1016/j.physbeh.2016.11.016.

André, C., Dinel, A.-L., Ferreira, G., Layé, S., Castanon, N., 2014. Diet-induced obesity progressively alters cognition, anxiety-like behavior and lipopolysaccharide-induced depressive-like behavior: focus on brain indoleamine 2,3-dioxygenase activation. Brain Behav. Immun. 41, 10–21. https://doi.org/10.1016/j.bbi.2014.03.012.

Barron, A.M., Rosario, E.R., Elteriefi, R., Pike, C.J., 2013. Sex-specific effects of high fat diet on indices of metabolic syndrome in 3xTg-AD mice: implications for Alzheimer's disease. PLoS One 8, e78554. https://doi.org/10.1371/journal.pone.0078554.

Bäuerl, C., Collado, M.C., Diaz Cuevas, A., Viña, J., Pérez Martínez, G., 2018. Shifts in gut microbiota composition in an APP/PSS1 transgenic mouse model of Alzheimer's disease during lifespan. Lett. Appl. Microbiol. 66, 464–471. https://doi.org/10.1111/lam.12882.

Benzing, W.C., Wujek, J.R., Ward, E.K., Shaffer, D., Ashe, K.H., Younkin, S.G., Brunden, K.R., 1999. Evidence for glial-mediated inflammation in aged APP(SW) transgenic mice. Neurobiol. Aging 20, 581–589. https://doi.org/10.1016/s0197-4580(99)00065-2.

Biyong, E.F., Alfos, S., Dumetz, F., Helbling, J.C., Aubert, A., Brossaud, J., Foury, A., Moisan, M.P., Layé, S., Richard, E., Patterson, E., Murphy, K., Rea, K., Stanton, C., Schellekens, H., Cryan, J.F., Capuron, L., Pallet, V., Ferreira, G., 2021. Dietary

vitamin a supplementation prevents early obesogenic diet-induced microbiota, neuronal and cognitive alterations. Int. J. Obes. 45, 588–598. https://doi.org/10.1038/s41366-020-00723-z.
Bridgewater, L.C., Zhang, C., Wu, Y., Hu, W., Zhang, Q., Wang, J., Li, S., Zhao, L., 2017. Gender-based differences in host behavior and gut microbiota composition in response to high fat diet and stress in a mouse model. Sci. Rep. 7, 10776. https://doi.org/10.1038/s41598-017-11069-4.
Burcelin, R., 2016. Gut microbiota and immune crosstalk in metabolic disease. Mol. Metab. 5, 771–781. https://doi.org/10.1016/j.molmet.2016.05.016.
Callahan, M.J., Lipinski, W.J., Bian, F., Durham, R.A., Pack, A., Walker, L.C., 2001. Augmented senile plaque load in aged female beta-amyloid precursor protein-transgenic mice. Am. J. Pathol. 158, 1173–1177. https://doi.org/10.1016/s0002-9440(10)64064-3.
Cani, P.D., Everard, A., 2016. Talking microbes: when gut bacteria interact with diet and host organs. Mol. Nutr. Food Res. 60, 58–66. https://doi.org/10.1002/mnfr.201500406.
Cani, P.D., Amar, J., Iglesias, M.A., Poggi, M., Knauf, C., Bastelica, D., Neyrinck, A.M., Fava, F., Tuohy, K.M., Chabo, C., Waget, A., Delmée, E., Cousin, B., Sulpice, T., Chamontin, B., Ferrières, J., Tanti, J.F., Gibson, G.R., Casteilla, L., Delzenne, N.M., Alessi, M.C., Burcelin, R., 2007. Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes. 56, 1761–1772. https://doi.org/10.2337/db06-1491.
Cao, D., Lu, H., Lewis, T.L., Li, L., 2007. Intake of sucrose-sweetened water induces insulin resistance and exacerbates memory deficits and amyloidosis in a transgenic mouse model of Alzheimer disease. J. Biol. Chem. 282, 36275–36282. https://doi.org/10.1074/jbc.M703561200.
Carr, D.B., Goate, A., Phil, D., Morris, J.C., 1997. Current concepts in the pathogenesis of Alzheimer's disease. Am. J. Med. 103, 3S–10S. https://doi.org/10.1016/s0002-9343(97)00262-3.
Carroll, J.C., Rosario, E.R., Chang, L., Stanczyk, F.Z., Oddo, S., LaFerla, F.M., Pike, C.J., 2007. Progesterone and estrogen regulate Alzheimer-like neuropathology in female 3xTg-AD mice. J. Neurosci. 27, 13357–13365. https://doi.org/10.1523/JNEUROSCI.2718-07.2007.
Cattaneo, A., Cattane, N., Galluzzi, S., Provasi, S., Lopizzo, N., Festari, C., Ferrari, C., Guerra, U.P., Paghera, B., Muscio, C., Bianchetti, A., Volta, G.D., Turla, M., Cotelli, M.S., Gennuso, M., Prelle, A., Zanetti, O., Lussignoli, G., Mirabile, D., Bellandi, D., Gentile, S., Belotti, G., Villani, D., Harach, T., Bolmont, T., Padovani, A., Boccardi, M., Frisoni, G.B., INDIA-FBP Group, 2017. Association of brain amyloidosis with pro-inflammatory gut bacterial taxa and peripheral inflammation markers in cognitively impaired elderly. Neurobiol. Aging 49, 60–68. https://doi.org/10.1016/j.neurobiolaging.2016.08.019.
Claesson, M.J., Wang, Q., O'Sullivan, O., Greene-Diniz, R., Cole, J.R., Ross, R.P., O'Toole, P.W., 2010. Comparison of two next-generation sequencing technologies for resolving highly complex microbiota composition using tandem variable 16S rRNA gene regions. Nucleic Acids Res. 38, e200 https://doi.org/10.1093/nar/gkq873.
Colonna, M., Wang, Y., 2016. TREM2 variants: new keys to decipher Alzheimer disease pathogenesis. Nat. Rev. Neurosci. 17, 201–207. https://doi.org/10.1038/nrn.2016.7.
Cosimo Melcangi, R., Garcia-Segura, L.M., 2010. Sex-specific therapeutic strategies based on neuroactive steroids: in search for innovative tools for neuroprotection. Horm. Behav. 57, 2–11. https://doi.org/10.1016/j.yhbeh.2009.06.001.
Cox, L.M., Schafer, M.J., Sohn, J., Vincentini, J., Weiner, H.L., Ginsberg, S.D., Blaser, Martin J., 2019. Calorie restriction slows age-related microbiota changes in an Alzheimer's disease model in female mice. Sci. Rep. 9, 17904. https://doi.org/10.1038/s41598-019-54187-x.
Cryan, J.F., Dinan, T.G., 2012. Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. Nat. Rev. Neurosci. 13, 701–712. https://doi.org/10.1038/nrn3346.
Cryan, J.F., Mombereau, C., Vassout, A., 2005. The tail suspension test as a model for assessing antidepressant activity: review of pharmacological and genetic studies in mice. Neurosci. Biobehav. Rev. 29, 571–625. https://doi.org/10.1016/j.neubiorev.2005.03.009.
Cryan, J.F., O'Riordan, K.J., Sandhu, K., Peterson, V., Dinan, T.G., 2020. The gut microbiome in neurological disorders. Lancet Neurol. 19, 179–194. https://doi.org/10.1016/S1474-4422(19)30356-4.
Cuello, A.C., 2017. Early and late CNS inflammation in Alzheimer's disease: two extremes of a continuum? Trends Pharmacol. Sci. 38, 956–966. https://doi.org/10.1016/j.tips.2017.07.005.
Cuervo-Zanatta, D., Garcia-Mena, J., Perez-Cruz, C., 2021. Gut microbiota alterations and cognitive impairment are sexually dissociated in a transgenic mice model of Alzheimer's disease. J. Alzheimers Dis. 82 (s1), S195–S214. https://doi.org/10.3233/JAD-201367.
Cuyvers, E., Bettens, K., Philtjens, S., Van Langenhove, T., Gijselinck, I., van der Zee, J., Engelborghs, S., Vandenbulcke, M., Van Dongen, J., Geerts, N., et al., 2014. Investigating the role of rare heterozygous TREM2 variants in Alzheimer's disease and frontotemporal dementia. Neurobiol. Aging 35, e711–e729. https://doi.org/10.1016/j.neurobiolaging.2013.09.009.
Dickson, D.W., Lee, S.C., Mattiace, L.A., Yen, S.H., Brosnan, C., 1993. Microglia and cytokines in neurological disease, with special reference to AIDS and Alzheimer's disease. Glia 7, 75–83. https://doi.org/10.1002/glia.440070113.
Dowlati, Y., Herrmann, N., Swardfager, W., Liu, H., Sham, L., Reim, E.K., Lanctôt, K.L., 2010. A meta-analysis of cytokines in major depression. Biol. Psychiatry 67, 446–457. https://doi.org/10.1016/j.biopsych.2009.09.033.
Eidson, L.N., de Sousa Rodrigues, M.E., Johnson, M.A., Barnum, C.J., Duke, B.J., Yang, Y., Chang, J., Kelly, S.D., Wildner, M., Tesi, R.J., Tansey, M.G., 2019. Chronic psychological stress during adolescence induces sex-dependent adulthood inflammation, increased adiposity, and abnormal behaviors that are ameliorated by selective inhibition of soluble tumor necrosis factor with XPro1595. Brain Behav. Immun. 81, 305–316. https://doi.org/10.1016/j.bbi.2019.06.027.
Elhaik Goldman, S., Goez, D., Last, D., Naor, S., Liraz Zaltsman, S., Sharvit-Ginon, I., Atrakchi-Baranes, D., Shemesh, C., Twitto-Greenberg, R., Tsach, S., Lotan, R., Leikin-Frenkel, A., Shish, A., Mardor, Y., Schnaider Beeri, M., Cooper, I., 2018. High-fat diet protects the blood-brain barrier in an Alzheimer's disease mouse model. Aging Cell 17, e12818. https://doi.org/10.1111/acel.12818.
Erny, D., Hrabě de Angelis, A.L., Jaitin, D., Wieghofer, P., Staszewski, O., David, E., Keren-Shaul, H., Mahlakoiv, T., Jakobshagen, K., Buch, T., Schwierzeck, V., Utermöhlen, O., Chun, E., Garrett, W.S., McCoy, K.D., Diefenbach, A., Staeheli, P., Stecher, B., Amit, I., Prinz, M., 2015. Host microbiota constantly control maturation and function of microglia in the CNS. Nat. Neurosci. 18, 965–977. https://doi.org/10.1038/nn.4030.
Everard, A., Belzer, C., Geurts, L., Ouwerkerk, J.P., Druart, C., Bindels, L.B., Guiot, Y., Derrien, M., Muccioli, G.G., Delzenne, N.M., de Vos, W.M., Cani, P.D., 2013. Cross-talk between *Akkermansia muciniphila* and intestinal epithelium controls diet-induced obesity. Proc.Nat.Acad.Sci.U.S.A 110, 9066–9071. https://doi.org/10.1073/pnas.1219451110.
Ferretti, L., McCurry, S.M., Logsdon, R., Gibbons, L., Teri, L., 2001. Anxiety and Alzheimer's disease. J.Geriatr.Psychiatry.Neurol. 14, 52–58. https://doi.org/10.1177/089198870101400111.
Fisher, D.W., Bennett, D.A., Dong, H., 2018. Sexual dimorphism in predisposition to Alzheimer's disease. Neurobiol. Aging 70, 308–324. https://doi.org/10.1016/j.neurobiolaging. 2018.04.004.
Fitz, N.F., Cronican, A., Pham, T., Fogg, A., Fauq, A.H., Chapman, R., Lefterov, I., Koldamova, R., 2010. Liver X receptor agonist treatment ameliorates amyloid pathology and memory deficits caused by high-fat diet in APP23 mice. J. Neurosci. 30, 6862–6872. https://doi.org/10.1523/JNEUROSCI.1051-10.2010.
Fu, R., Shen, Q., Xu, P., Luo, J.J., Tang, Y., 2014. Phagocytosis of microglia in the central nervous system diseases. Mol. Neurobiol. 49, 1422–1434. https://doi.org/10.1007/s12035-013-8620-8626.
Fülling, C., Lach, G., Bastiaanssen, T.F.S., Fouhy, F., O'Donovan, A.N., Ventura-Silva, A. P., Stanton, C., Dinan, T.G., Cryan, J.F., 2020. Adolescent dietary manipulations differentially affect gut microbiota composition and amygdala neuroimmune gene expression in male mice in adulthood. Brain Behav. Immun. 87, 666–678. https://doi.org/10.1016/j.bbi.2020.02.013.
Gainey, S.J., Kwakwa, K.A., Bray, J.K., Pillote, M.M., Tir, V.L., Towers, A.E., Freund, G. G., 2016. Short-term high-fat diet (HFD) induced anxiety-like behaviors and cognitive impairment are improved with treatment by glyburide. Front. Behav. Neurosci. 10, 156. https://doi.org/10.3389/fnbeh.2016.00156.
Graham, L.C., Harder, J.M., Soto, I., de Vries, W.N., John, S.W., Howell, G.R., 2016. Chronic consumption of a western diet induces robust glial activation in aging mice and in a mouse model of Alzheimer's disease. Sci. Rep. 18 (6), 21568. https://doi.org/10.1038/srep21568.
Guillemot-Legris, O., Muccioli, G.G., 2017. Obesity-induced Neuroinflammation: beyond the hypothalamus. Trends Neurosci. 40, 237–253. https://doi.org/10.1016/j.tins.2017.02.005.
Guo, L.X., Tong, Y., Wang, J., Yin, G., Huang, H.S., Zeng, L., Wang, P., Li, J.P., Bi, K.S., Wang, T.J., 2020. Determination and comparison of short-chain fatty acids in serum and Colon content samples: Alzheimer's disease rat as a case study. Molecules. 25, 5739. https://doi.org/10.3390/molecules25235739.
Hansen, C.H.F., Krych, L., Nielsen, D.S., Vogensen, F.K., Hansen, L.H., Sørensen, S.J., Buschard, K., Hansen, A.K., 2012. Early life treatment with vancomycin propagates Akkermansia muciniphila and reduces diabetes incidence in the NOD mouse. Diabetologia. 55, 2285–2294. https://doi.org/10.1007/s00125-012-2564-7.
Harach, T., Marungruang, N., Duthilleul, N., Cheatham, V., Mc Coy, K.D., Frisoni, G., Neher, J.J., Fåk, F., Jucker, M., Lasser, T., Bolmont, T., 2017. Reduction of Abeta amyloid pathology in APPPS1 transgenic mice in the absence of gut microbiota. Sci. Rep. 7, 41802. https://doi.org/10.1038/srep41802.
Heneka, M.T., Carson, M., El Khoury, J., Landreth, G.E., Brosseron, F., Feinstein, D.L., et al., 2015. Neuroinflammation in Alzheimer's disease. Lancet Neurol. 14, 388–405. https://doi.org/10.1016/S1474-4422(15)70016-5.
Heppner, F.L., Ransohoff, R.M., Becher, B., 2015. Immune attack: the role of inflammation in Alzheimer disease. Nat. Rev. Neurosci. 16, 358–372. https://doi.org/10.1038/nrn3880.
Hickman, S.E., Allison, E.K., El Khoury, J., 2008. Microglial dysfunction and defective beta-amyloid clearance pathways in aging Alzheimer's disease mice. J. Neurosci. 28, 8354–8360. https://doi.org/10.1523/JNEUROSCI.0616-08.2008.
Holmes, C., Cunningham, C., Zotova, E., Woolford, J., Dean, C., Kerr, S., et al., 2009. Systemic inflammation and disease progression in Alzheimer disease. Neurology. 73, 768–774. https://doi.org/10.1212/WNL.0b013e3181b6bb95.
Holmes, C., Cunningham, C., Zotova, E., Culliford, D., Perry, V.H., 2011. Proinflammatory cytokines, sickness behavior, and Alzheimer disease. Neurology 77, 212–218. https://doi.org/10.1212/WNL.0b013e318225ae07.
Husemann, J., Loike, J.D., Anankov, R., Febbraio, M., Silverstein, S.C., 2002. Scavenger receptors in neurobiology and neuropathology: their role on microglia and other cells of the nervous system. Glia 40, 195–205. https://doi.org/10.1002/glia.10148.
Hwang, L.L., Wang, C.H., Li, T.L., Chang, S.D., Lin, L.C., Chen, C.P., Chen, C.T., Liang, K. C., Ho, I.K., Yang, W.S., Chiou, L.C., 2010. Sex differences in high-fat diet-induced obesity, metabolic alterations and learning, and synaptic plasticity deficits in mice. Obesity (Silver Spring) 18, 463–469. https://doi.org/10.1038/oby.2009.273.
Ingvorsen, C., Karp, N.A., Lelliott, C.J., 2017. The role of sex and body weight on the metabolic effects of high-fat diet in C57BL/6N mice. Nutr. Diabetes. 7, e261 https://doi.org/10.1038/nutd.2017.6.

Int Veld, B., Ruitenberg, A., Hofman, A.L., Launer, L.J., van Duijn, C.M., Stijnen, T., Breteler, M.M., Stricker, B.H., 2001. Nonsteroidal antiinflammatory drugs and the risk of Alzheimer's disease. N. Engl. J. Med. 345, 1515–1521. https://doi.org/10.1056/NEJMoa010178.

Jaggar, M., Rea, K., Spichak, S., Dinan, T.G., Cryan, J.F., 2020. You've got male: sex and the microbiota-gut-brain axis across the lifespan. Front. Neuroendocrinol. 56, 100815. https://doi.org/10.1016/j.yfrne.2019.100815.

Jayaraman, A., Lent-Schochet, D., Pike, C.J., 2014. Diet-induced obesity and low testosterone increase neuroinflammation and impair neural function. J. Neuroinflammation 16 (11), 162. https://doi.org/10.1186/s12974-014-0162-y.

Julien, C., Tremblay, C., Phivilay, A., Berthiaume, L., Emond, V., Julien, P., Calon, F., 2010. High-fat diet aggravates amyloid-beta and tau pathologies in the 3xTg-AD mouse model. Neurobiol. Aging 31, 1516–1531. https://doi.org/10.1016/j.neurobiolaging.2008.08.022.

Karlsson, C.L.J., Onnerfält, J., Xu, J., Molin, G., Ahrné, S., Thorngren-Jerneck, K., 2012. The microbiota of the gut in preschool children with normal and excessive body weight. Obesity (Silver Spring) 20, 2257–2261. https://doi.org/10.1038/oby.2012.110.

Kaur, T., Kaur, G., 2017. Withania somnifera as a potential candidate to ameliorate high fat diet-induced anxiety and neuroinflammation. J. Neuroinflammation 14, 201. https://doi.org/10.1186/s12974-017-0975-6.

Kaur, H., Golovko, S., Golovko, M.Y., Singh, S., Darland, D.C., Combs, C.K., 2020. Effects of probiotic supplementation on short chain fatty acids in the AppNL-G-F mouse model of Alzheimer's disease. J. Alzheimers Dis. 76, 1083–1102. https://doi.org/10.3233/JAD-200436.

Kim, D.G., Krenz, A., Toussaint, L.E., Maurer, K.J., Robinson, S., Yan, A., et al., 2016. Non-alcoholic fatty liver disease induces signs of Alzheimer's disease (AD) in wild-type mice and accelerates pathological signs of AD in an AD model. J. Neuroinflammation 13, 1. https://doi.org/10.1186/s12974-015-0467-5.

Kivipelto, M., Ngandu, T., Fratiglioni, L., Viitanen, M., Kåreholt, I., Winblad, B., et al., 2005. Obesity and vascular risk factors at midlife and the risk of dementia and Alzheimer disease. Arch. Neurol. 62, 1556–1560. https://doi.org/10.1001/archneur.62.10.1556.

Knight, E.M., Martins, I.V., Gümüsgöz, S., Allan, S.M., Lawrence, C.B., 2014. High-fat diet-induced memory impairment in triple-transgenic Alzheimer's disease (3xTgAD) mice is independent of changes in amyloid and tau pathology. Neurobiol. Aging 35, 1821–1832. https://doi.org/10.1016/j.neurobiolaging.2014.02.010.

Kohjima, M., Sun, Y., Chan, L., 2010. Increased food intake leads to obesity and insulin resistance in the tg2576 Alzheimer's disease mouse model. Endocrinology. 151, 1532–1540. https://doi.org/10.1210/en.2009-1196.

Kong, C.-Y., Li, Z.-M., Han, B., Zhang, Z.-Y., Chen, H.-L., Zhang, S.-L., Xu, J.-Q., Mao, Y.-Q., Zhao, Y.-P., Wang, L.-S., 2019. Diet consisting of balanced yogurt, fruit, and vegetables modifies the gut microbiota and protects mice against nonalcoholic fatty liver disease. Mol. Nutr. Food Res. 63 (19) https://doi.org/10.1002/mnfr.201900249 e1900249.

Lee, K.W., Im, J.Y., Song, J.S., Lee, S.H., Lee, H.J., Ha, H.Y., Koh, J.Y., Gwag, B.J., Yang, S.D., Paik, S.G., Han, P.L., 2006. Progressive neuronal loss and behavioral impairments of transgenic C57BL/6 inbred mice expressing the carboxy terminus of amyloid precursor protein. Neurobiol. Dis. 22, 10–24. https://doi.org/10.1016/j.nbd.2005.09.011.

Leigh, S.J., Kaakoush, N.O., Bertoldo, M.J., Westbrook, R.F., Morris, M.J., 2020. Intermittent cafeteria diet identifies fecal microbiome changes as a predictor of spatial recognition memory impairment in female rats. Transl. Psychiatry 10, 36. https://doi.org/10.1038/s41398-020-0734-9.

Liu, D., Wang, Z., Liu, S., Wang, F., Zhao, S., Hao, A., 2011. Anti-inflammatory effects of fluoxetine in lipopolysaccharide(LPS)-stimulated microglial cells. Neuropharmacology. 61, 592–599. https://doi.org/10.1016/j.neuropharm.2011.04.033.

Liu, P., Wu, L., Peng, G., Han, Y., Tang, R., Ge, J., Zhang, L., Jia, L., Yue, S., Zhou, K., Li, L., Luo, B., Wang, B., 2019. Altered microbiomes distinguish Alzheimer's disease from amnestic mild cognitive impairment and health in a Chinese cohort. Brain Behav. Immun. 80, 633–643. https://doi.org/10.1016/j.bbi.2019.05.008.

Lyketsos, C.G., Lopez, O., Jones, B., Fitzpatrick, A.L., Breitner, J., DeKosky, S., 2002. Prevalence of neuropsychiatric symptoms in dementia and mild cognitive impairment: results from the cardiovascular health study. JAMA. 288, 1475–1483. https://doi.org/10.1001/jama.288.12.1475.

Maes, M., Yirmyia, R., Noraberg, J., Brene, S., Hibbeln, J., Perini, G., Kubera, M., Bob, P., Lerer, B., Maj, M., 2009. The inflammatory & neurodegenerative (I&ND) hypothesis of depression: leads for future research and new drug developments in depression. Metab. Brain Dis. 24, 27–53. https://doi.org/10.1007/s11011-008-9118-1.

Maesako, M., Uemura, K., Kubota, M., Kuzuya, A., Sasaki, K., Asada, M., Watanabe, K., Hayashida, N., Ihara, M., Ito, H., Shimohama, S., Kihara, T., Kinoshita, A., 2012a. Environmental enrichment ameliorated high-fat diet-induced Aβ deposition and memory deficit in APP transgenic mice. Neurobiol. Aging 33, 1011 e11-23. https://doi.org/10.1016/j.neurobiolaging.2011.10.028.

Maesako, M., Uemura, K., Kubota, M., Kuzuya, A., Sasaki, K., Hayashida, N., Asada-Utsugi, M., Watanabe, K., Uemura, M., Kihara, T., Takahashi, R., Shimohama, S., Kinoshita, A., 2012b. Exercise is more effective than diet control in preventing high fat diet-induced β-amyloid deposition and memory deficit in amyloid precursor protein transgenic mice. J. Biol. Chem. 287, 23024–23033. https://doi.org/10.1074/jbc.M112.367011.

Malik, V.S., Willett, W.C., Hu, F.B., 2013. Global obesity: trends, risk factors and policy implications. Nat. Rev. Endocrinol. 9, 13–27. https://doi.org/10.1038/nrendo.2012.199.

Martin, A.A., Davidson, T.L., 2014. Human cognitive function and the obesogenic environment. Physiol. Behav. 136, 185–193. https://doi.org/10.1016/j.physbeh.2014.02.062.

Mazure, C.M., Swendsen, J., 2016. Sex differences in Alzheimer's disease and other dementias. Lancet Neurol. 15, 451–452. https://doi.org/10.1016/S1474-4422(16)00067-3.

McGeer, P.L., Schulzer, M., McGeer, E.G., 1996. Arthritis and anti-inflammatory agents as possible protective factors for Alzheimer's disease: a review of 17 epidemiologic studies. Neurology 47, 425–432. https://doi.org/10.1212/wnl.47.2.425.

McKenzie, J.A., Spielman, L.J., Pointer, C.B., Lowry, J.R., Bajwa, E., Lee, C.W., et al., 2017. Neuroinflammation as a common mechanism associated with the modifiable risk factors for Alzheimer's and Parkinson's diseases. Curr. Aging Sci. https://doi.org/10.2174/1874609810666170315113244.

Minter, M.R., Hinterleitner, R., Meisel, M., Zhang, C., Leone, V., Zhang, X., Oyler-Castrillo, P., Zhang, X., Musch, M.W., Shen, X., Jabri, B., Chang, E.B., Tanzi, R.E., Sisodia, S.S., 2017. Antibiotic-induced perturbations in microbial diversity during post-natal development alters amyloid pathology in an aged APPSWE/PS1ΔE9 murine model of Alzheimer's disease. Sci. Rep. 7, 10411. https://doi.org/10.1038/s41598-017-11047-w.

Morrison, K.E., Jašarević, E., Howard, C.D., Bale, T.L., 2020. It's the fiber, not the fat: significant effects of dietary challenge on the gut microbiome. Microbiome. 8, 15. https://doi.org/10.1186/s40168-020-0791-6.

Murphy, E.F., Cotter, P.D., Healy, S., Marques, T.M., O'Sullivan, O., Fouhy, F., Clarke, S.F., O'Toole, P.W., Quigley, E.M., Stanton, C., Ross, P.R., O'Doherty, R.M., Shanahan, F., 2010. Composition and energy harvesting capacity of the gut microbiota: relationship to diet, obesity and time in mouse models. Gut 59, 1635–1642. https://doi.org/10.1136/gut.2010.215665.

Murphy, E.A., Velazquez, K.T., Herbert, K.M., 2015. Influence of high-fat diet on gut microbiota: a driving force for chronic disease risk. Curr Opin Clin Nutr Metab Care. 18, 515–520. https://doi.org/10.1097/MCO.0000000000000209.

Nedungadi, T.P., Clegg, D.J., 2009. Sexual dimorphism in body fat distribution and risk for cardiovascular diseases. J. Cardiovasc. Transl. Res. 2, 321–327. https://doi.org/10.1007/s12265-009-9101-1.

Ou, Z., Deng, L., Lu, Z., Wu, F., Liu, W., Huang, D., Peng, Y., 2020. Protective effects of Akkermansia muciniphila on cognitive deficits and amyloid pathology in a mouse model of Alzheimer's disease. Nutr Diabetes 10, 12. https://doi.org/10.1038/s41387-020-0115-8.

Paganini-Hill, A., Henderson, V.W., 1994. Estrogen deficiency and risk of Alzheimer's disease in women. Am. J. Epidemiol. 140, 256–261. https://doi.org/10.1093/oxfordjournals.aje.a117244.

Pappolla, M.A., Perry, G., Fang, X., Zagorski, M., Sambamurti, K., Poeggeler, B., 2021. Indoles as essential mediators in the gut-brain axis. Their role in Alzheimer's disease. Neurobiol Dis. Aug 156. https://doi.org/10.1016/j.nbd.2021.105403, 105403.

Pasinetti, G.M., Eberstein, J.A., 2008. Metabolic syndrome and the role of dietary lifestyles in Alzheimer's disease. J. Neurochem. 106, 1503–1514. https://doi.org/10.1111/j.1471-4159.2008.05454.x.

Patel, N.S., Paris, D., Mathura, V., Quadros, A.N., Crawford, F.C., Mullan, M.J., 2005. Inflammatory cytokine levels correlate with amyloid load in transgenic mouse models of Alzheimer's disease. J. Neuroinflammation 2, 9. https://doi.org/10.1186/1742-2094-2-9.

Perez-Nievas, B.G., Stein, T.D., Tai, H.C., Dols-Icardo, O., Scotton, T.C., Barroeta-Espar, I., Fernandez-Carballo, L., de Munain, E.L., Perez, J., Marquie, M., Serrano-Pozo, A., Frosch, M.P., Lowe, V., Parisi, J.E., Petersen, R.C., Ikonomovic, M.D., López, O.L., Klunk, W., Hyman, B.T., Gómez-Isla, T., 2013. Dissecting phenotypic traits linked to human resilience to Alzheimer's pathology. Brain. 136, 2510–2526. https://doi.org/10.1093/brain/awt171.

Pfaffl, M.W., Tichopad, A., Prgomet, C., Neuvians, T.P., 2004. Determination of stable housekeeping genes, differentially regulated target genes and sample integrity: BestKeeper–excel-based tool using pair-wise correlations. Biotechnol. Lett. 26, 509–515. https://doi.org/10.1023/b:bile.0000019559.84305.47.

Pike, C.J., 2017. Sex and the development of Alzheimer's disease. J. Neurosci. Res. 95, 671–680. https://doi.org/10.1002/jnr.23827.

Pike, C.J., Carroll, J.C., Rosario, E.R., Barron, A.M., 2009. Protective actions of sex steroid hormones in Alzheimer's disease. Front. Neuroendocrinol. 30, 239–258. https://doi.org/10.1016/j.yfrne.2009.04.015.

Power, M.L., Schulkin, J., 2008. Sex differences in fat storage, fat metabolism, and the health risks from obesity: possible evolutionary origins. Br. J. Nutr. 99, 931–940. https://doi.org/10.1017/S0007114507853347.

Sala Frigerio, C., Wolfs, L., Fattorelli, N., Thrupp, N., Voytyuk, I., Schmidt, I., Mancuso, R., Chen, W.T., Woodbury, M.E., Srivastava, G., Möller, T., Hudry, E., Das, S., Saido, T., Karran, E., Hyman, B., Perry, V.H., Fiers, M., De Strooper, B., 2019. The major risk factors for Alzheimer's disease: age, sex, and genes modulate the microglia response to Aβ plaques. Cell Rep. 27, 1293–1306 e6. https://doi.org/10.1016/j.celrep.2019.03.099.

Sharma, S., Fulton, S., 2013. Diet-induced obesity promotes depressive-like behaviour that is associated with neural adaptations in brain reward circuitry. Int. J. Obes. 37, 382–389.

Shi, Y., Yue, F., Xing, L., Wu, S., Wei, L., Xiang, X., Russell, R., Xia, K., Zhang, D., 2020. Sex differences in gut microbiome in high-fat-diet fed rats. Preprint from Research Square. https://doi.org/10.21203/rs.3.rs-98831/v1.

Smith, E., Hay, P., Campbell, L., Trollor, J.N., 2011. A review of the association between obesity and cognitive function across the lifespan: implications for novel approaches to prevention and treatment. Obes. Rev. 12, 740–755. https://doi.org/10.1111/j.1467-789X.2011.00920.x.

Tramunt, B., Smati, S., Grandgeorge, N., Lenfant, F., Arnal, J.-F., Montagner, A., Pierre Gourdy, P., 2020. Sex differences in metabolic regulation and diabetes susceptibility. Diabetologia 63, 453–461. https://doi.org/10.1007/s00125-019-05040-3.

Tucsek, Z., Toth, P., Sosnowska, D., Gautam, T., Mitschelen, M., Koller, A., et al., 2014. Obesity in aging exacerbates blood-brain barrier disruption, Neuroinflammation, and oxidative stress in the mouse Hippocampus: effects on expression of genes involved in Beta-amyloid generation and Alzheimer's disease. J. Gerontol. A Biol. Sci. Med. Sci. 69, 1212–1226. https://doi.org/10.1093/gerona/glt177.

Vogt, N.M., Kerby, R.L., Dill-McFarland, K.A., Harding, S.J., Merluzzi, A.P., Johnson, S. C., Carlsson, C.M., Asthana, S., Zetterberg, H., Blennow, K., Bendlin, B.B., Rey, F.E., 2017. Gut microbiome alterations in Alzheimer's disease. Sci. Rep. 7 (1), 13537. https://doi.org/10.1038/s41598-017-13601-y. Oct 19.

Whitmer, R.A., Quesenberry, C.P., Zhou, J., Yaffe, K., 2011. Timing of hormone therapy and dementia: the critical window theory revisited. Ann. Neurol. 69 (1), 163–169. https://doi.org/10.1002/ana.22239.

Winter, S.E., Bäumler, A.J., 2014. Dysbiosis in the inflamed intestine: chance favors the prepared microbe. Gut Microbes 5, 71–73. https://doi.org/10.4161/gmic.27129.

Wu, G.D., Chen, J., Hoffmann, C., Bittinger, K., Chen, Y.-Y., Keilbaugh, S.A., Bewtra, M., Knights, D., Walters, W.A., Knight, R., Sinha, R., Gilroy, E., Gupta, K., Baldassano, R., Nessel, Lisa, Li, H., Bushman, F.D., Lewis, J.D., 2011. Linking long-term dietary patterns with gut microbial Enterotypes. Science 334, 105–108. https://doi.org/10.1126/science.1208344.

Wyss-Coray, T., 2006. Inflammation in Alzheimer disease: driving force, bystander or beneficial response? Nat. Med. 12, 1005–1015. https://doi.org/10.1038/nm1484.

Xu, N., Li, X., Zhong, Y., 2015. Inflammatory cytokines: potential biomarkers of immunologic dysfunction in autism spectrum disorders. Mediat. Inflamm. 531518 doi:531510.1155/2015/531518.

Xu, M., Mo, X., Huang, H., Chen, X., Liu, H., Peng, Z., Chen, L., Rong, S., Yang, W., Xu, S., Liu, L., 2020. Yeast β-glucan alleviates cognitive deficit by regulating gut microbiota and metabolites in Aβ1-42-induced AD-like mice. Int. J. Biol. Macromol. 161, 258–270. https://doi.org/10.1016/j.ijbiomac.2020.05.180. Oct 15.

Yirmiya, R., Pollak, Y., Barak, O., Avitsur, R., Ovadia, H., Bette, M., Weihe, E., Weidenfeld, J., 2001. Effects of antidepressant drugs on the behavioral and physiological responses to lipopolysaccharide (LPS) in rodents. Neuropsychopharmacol. 24, 531–544.

Zhao, L., Mao, Z., Chen, S., Schneider, L.S., Brinton, R.D., 2013. Early intervention with an estrogen receptor β-selective phytoestrogenic formulation prolongs survival, improves spatial recognition memory, and slows progression of amyloid pathology in a female mouse model of Alzheimer's disease. J. Alzheimers Dis. 37, 403–419. https://doi.org/10.3233/JAD-122341.

Zhuang, Z.Q., Shen, L.L., Li, W.W., Fu, X., Zeng, F., Gui, L., Lü, Y., Cai, M., Zhu, C., Tan, Y.L., Zheng, P., Li, H.Y., Zhu, J., Zhou, H.D., Bu, X.L., Wang, Y.J., 2018. Gut microbiota is altered in patients with Alzheimer's disease. J. Alzheimers Dis. 63, 1337–1346. https://doi.org/10.3233/JAD-180176.

Zore, T., Palafox, M., Reue, K., 2018. Sex differences in obesity, lipid metabolism, and inflammation-a role for the sex chromosomes? Mol. Metab. 15, 35–44. https://doi.org/10.1016/j.molmet.2018.04.003.