Distribution Agreement

In presenting this thesis as a partial fulfillment of the requirements for a degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis in whole or in part in all forms of media, now or hereafter now, including display on the World Wide Web. I understand that I may select some access restrictions as part of the online submission of this thesis. I retain all ownership rights to the copyright of the thesis. I also retain the right to use in future works (such as articles or books) all or part of this thesis.

Noah Schwartz April 10, 2023

Impact of Genotype and High-Fat High-Carbohydrate Diet on the Colon in the TgF344-AD and 5xFAD Rodent Models

by

Noah Schwartz

Timothy Sampson, PhD.
Adviser

Neuroscience and Behavioral Biology

Timothy Sampson, PhD.

Adviser

David Weinshenker, PhD.

Committee Member

Kristen Frenzel, PhD.

Committee Member

2023

Impact of Genotype and High-Fat High-Carbohydrate Diet on the Colon in the TgF344-AD and 5xFAD Rodent Models

By

Noah Schwartz

Timothy Sampson, PhD.

Adviser

An abstract of
a thesis submitted to the Faculty of Emory College of Arts and Sciences
of Emory University in partial fulfillment
of the requirements of the degree of
Bachelor of Science with Honors

Neuroscience and Behavioral Biology

2023

Abstract

Impact of Genotype and High-Fat High-Carbohydrate Diet on the Colon in the TgF344-AD and 5xFAD Rodent Models

By Noah Schwartz

Background

Elevated levels of soluble amyloid beta, accumulation of amyloid plaques, neuronal cell death, and inflammation are main features of Alzheimer's disease (AD) pathology in the central nervous system (CNS). While the presentation of AD in the CNS is well characterized, a gap in understanding exists in how AD pathology displays in the enteric nervous system (ENS). The literature supports peripheral chronic inflammation contribution to the exacerbation of brain AD pathology, but little is known about the specific role of the ENS in the onset and progression of AD.

Methods

Here we investigated how the genotype for AD and high-fat high-carbohydrate diet (HFHC) intake interacts to impact the ENS. The colon tissue of 5xFAD female mice (2 months of age) and TgF344-AD female and male rats (6 and 15 months of age) were assessed with immunoassays and western botting to evaluate the presence of genotype-dependent hallmarks of AD identified in the brain including: soluble amyloid, key neuronal marker protein expression, and inflammation. For genotype-diet interactions, 5xFAD mice were fed a HFHC diet for 8 weeks to investigate the impact of a known inflammation-inducing environmental factor on AD pathology in the intestine. Behavioral tests for fecal transit were performed.

Results

The colon of the 5xFAD female mice did not present detectable soluble amyloid in the colon at 4 months of age, suggesting diet-genotype interaction did not impact the soluble amyloid concentrations in the colon. The TgF344-AD rat model presented intestinal soluble amyloid. TgF344-AD rats also demonstrate an age-dependent increase in interferon gamma (IFN-γ, a proinflammatory cytokine shown to contribute to increases in amyloid concentrations in the CNS). HFHC diet regulates inflammatory markers IL-1β, CXCL1, and IL-5 in 5xFAD mice. Additionally, a decrease in fecal water content was observed in HFHC-fed 5xFAD mice.

Conclusions

Our novel findings support a genotype effect on AD pathology in the intestine of the TgF344-AD rat model. While the mechanisms contributing to presentation of AD pathology in the ENS require future studies to define, possible factors include the unique AD-linked mutations and the mouse prion promotor driving expression of APP and PSEN1 genes in the transgenic rats.

Our findings in the 5xFAD mouse model suggest that a HFHC diet may modulate inflammation of the intestine in a manner independent of amyloid production. Additionally, HFHC diet contributes to constipation, a symptom of GI disorders frequently associated with intestinal inflammation. Collectively, our findings are an important step to improving our understanding of intestinal AD pathology and how it impacts the disease overall.

Keywords: Amyloid beta, Enteric nervous system, Peripheral inflammation, Intestine, Colon

Impact of Genotype and High-Fat High-Carbohydrate Diet on the Colon in the TgF344-AD and 5xFAD Rodent Models

By

Noah Schwartz

Timothy Sampson, PhD.

Adviser

A thesis submitted to the Faculty of Emory College of Arts and Sciences
of Emory University in partial fulfillment
of the requirements of the degree of
Bachelor of Science with Honors

Neuroscience and Behavioral Biology

2023

Acknowledgements

I thank Dr. Timothy Sampson for approving and guiding my thesis research, Dr. Elizabeth Rodrigues for training me in laboratory techniques, and all the members of the Sampson Lab for their support in completion of my thesis work. Additional thanks to Dr. David Weinshenker and Dr. Kristen Frenzel for serving on my honors committee, and to the Weinshenker lab for providing the rat colon tissue used in the experiments.

Table of Contents

Abstract

Background

Elevated levels of soluble amyloid beta, accumulation of amyloid plaques, neuronal cell death, and inflammation are main features of Alzheimer's disease (AD) pathology in the central nervous system (CNS). While the presentation of AD in the CNS is well characterized, a gap in understanding exists in how AD pathology displays in the enteric nervous system (ENS). The literature supports peripheral chronic inflammation contribution to the exacerbation of brain AD pathology, but little is known about the specific role of the ENS in the onset and progression of AD.

Methods

Here we investigated how the genotype for AD and high-fat high-carbohydrate diet (HFHC) intake interacts to impact the ENS. The colon tissue of 5xFAD female mice (2 months of age) and TgF344-AD female and male rats (6 and 15 months of age) were assessed with immunoassays and western botting to evaluate the presence of genotype-dependent hallmarks of AD identified in the brain including: soluble amyloid, key neuronal marker protein expression, and inflammation. For genotype-diet interactions, 5xFAD mice were fed a HFHC diet for 8 weeks to investigate the impact of a known inflammation-inducing environmental factor on AD pathology in the intestine. Behavioral tests for fecal transit were performed.

Results

The colon of the 5xFAD female mice did not present detectable soluble amyloid in the colon at 4 months of age, suggesting diet-genotype interaction did not impact the soluble amyloid concentrations in the colon. The TgF344-AD rat model presented intestinal soluble amyloid.

TgF344-AD rats also demonstrate an age-dependent increase in interferon gamma (IFN-γ, a proinflammatory cytokine shown to contribute to increases in amyloid concentrations in the CNS). HFHC diet regulates inflammatory markers IL-1β, CXCL1, and IL-5 in 5xFAD mice. Additionally, a decrease in fecal water content was observed in HFHC-fed 5xFAD mice.

Conclusions

Our novel findings support a genotype effect on AD pathology in the intestine of the TgF344-AD rat model. While the mechanisms contributing to presentation of AD pathology in the ENS require future studies to define, possible factors include the unique AD-linked mutations and the mouse prion promotor driving expression of APP and PSEN1 genes in the transgenic rats.

Our findings in the 5xFAD mouse model suggest that a HFHC diet may modulate inflammation of the intestine in a manner independent of amyloid production. Additionally, HFHC diet contributes to constipation, a symptom of GI disorders frequently associated with intestinal inflammation. Collectively, our findings are an important step to improving our understanding of intestinal AD pathology and how it impacts the disease overall.

Keywords: Amyloid beta, Enteric nervous system, Peripheral inflammation, Intestine, Colon

Introduction

Alzheimer's disease (AD) is a neurodegenerative condition defined by a progressive loss of memory and the cognitive functions associated with planning, reasoning, and problem-solving (McGirr et al., 2020). AD is the most common cause of dementia, and its prevalence worldwide is approximately 24 million people (Alzheimer's Association, 2021). Estimates suggest that roughly 6 million Americans live with AD currently, and that number is projected to increase to 14 million people by the year 2060 (Matthews et al., 2019). For the 65+ year old population, the number of people with AD doubles every 5 years (Alzheimer's Association, 2021). AD is the sixth leading cause of death in the United States and the estimated annual cost attributed to the care of Americans with AD is 355 billion dollars (Alzheimer's Association, 2021). Despite the major negative outcomes associated with people suffering from AD, there are still no effective treatments to prevent, slow, or cure this neurodegenerative disease.

There are several major hallmarks of AD pathology. These include: 1) abnormally increased deposition of amyloid beta (Aβ) and subsequent aggregation of extracellular amyloid-beta plaques in the brain 2), neuronal cell dysfunction and death, 3) abnormal deposits of Tau protein as neurofibrillary tangles inside neurons and 4) neuroinflammation (Kumfor et al., 2017).

The deposition of increased (relative to baseline) concentrations of Aβ in the brain which lead to the formation of insoluble plaques is a distinct pathology of AD. While healthy individuals do produce Aβ - the protein is associated with important biological functions including hormone storage (Greenwald and Riek, 2010; Fowler et al., 2007) – the amyloid hypothesis suggests that Aβ protein misfolding into senile plaques in the CNS is the main cause of AD (Haass and Selkoe, 1993). Expression of the amyloid precursor protein (APP) gene enables synthesis of the protein product APP. This protein has regulatory functions associated with neural plasticity,

synapse formation, and antimicrobial activity. APP undergoes sequential cleaving by proteolytic enzymes (first β-secretase then γ-secretase). Presenilin is a protein that acts as a catalytic component of the γ-secretase complex which cleaves a form of APP to ultimately produce Aβ peptides (De Strooper et al., 2012). In the context of AD pathology, the increased ratio of two of these cleaved peptides (Aβ42 over Aβ40) is correlated with the onset of the disease (Dang et al., 2015).

There is also evidence for Aβ driving the inflammatory mechanisms involved in AD (Meraz-Ríos et al., 2013). Aβ deposition precipitates an acute immune response of glial cells (microglia and astrocytes) resulting in the increased production of proinflammatory cytokines (e.g. TNF, IL-1, IL-6) which is an indicator of immune and glial cell activation. These molecules contribute to a chronic activation state in immune cells and can increase amyloid production via signaling for increased secretase cleavage of APP, resulting in a cycle of escalation of the inflammatory response. Accumulation of amyloid into plaques indirectly activates caspases that produce proinflammatory cytokines which amplify plaque-induced neuroinflammation (Meraz-Ríos et al., 2013).

Current data suggest that Aβ pathology is associated with neuronal cell dysfunction and that the accumulation of plaques in the cerebral cortex and hippocampus increase neuroinflammation, which influences cell death (Meraz-Ríos et al., 2013; Leong et al., 2020). Cell death (apoptosis) primarily occurs through either an intrinsic or extrinsic pathway (Hengartner, 2000). The intrinsic pathway involves apoptotic proteins targeting the mitochondria, the organelle involved in aerobic cellular respiration that when damaged or destroyed results in rapid cell death. The extrinsic pathway involves increased production of TNF, a proinflammatory cytokine that regulates apoptosis. Both pathways are implicated in the neuronal apoptosis associated with AD,

and APP functions differently in each one. Overexpression of APP elicits the intracellular stress that initiates the intrinsic pathway. In the extrinsic pathway, APP and Aβ peptides bind to a cell surface receptor (DR6) belonging to the TNF receptor superfamily that leads to activation of caspases and subsequent apoptosis (Leong et al., 2020).

Shared genes between AD and GI disorders have been found (Santiago and Potashkin, 2021) indicating an association between AD pathology and that of some intestinal diseases. Expression of several of these co-occurring genes encode proteins that regulate cellular processes including signal transduction (PDE4B), senescence (PHB), and inhibition of proliferation (SCARA3). While the effects of AD on the gastrointestinal (GI) tract are not fully understood, one common symptom of AD patients is incontinence which indicates that AD does affect normal GI tract function. Conversely, the physiological status of the GI tract can impact AD pathology in the CNS. One mechanism by which the GI tract exerts its effect on the brain involves increased permeability of the intestinal epithelium enabling increased movement of metabolites, including inflammatory bacterial products, from the intestinal lumen into circulation. These can signal through or across the blood-brain-barrier (BBB) and stimulate immune cells towards an inflammatory response resulting in proinflammatory cytokine production. Movement of specific metabolites and cytokines across BBB can promote amyloid plaque formation (via increased APP expression) and neuroinflammation, thus impairing CNS function and increasing risk of AD (Meraz-Ríos et al., 2013; Kinney et al., 2018).

The GI tract can also be impacted by the immune dysregulation present in obesity. One of the hallmarks of obesity is chronic low-grade systemic inflammation defined by upregulation of proinflammatory cytokine secretion from adipose cells which stimulates immune cells and initiates an inflammatory response (Alford et al., 2018). Thus, systemic inflammation serves as a

central link between obesity and AD. Neuroinflammation is marked by increased proinflammatory cytokine production by microglia of the CNS, but enhanced inflammatory cytokine production in the periphery can result in increased influx of inflammatory markers across the BBB, compounding the local inflammatory response in the brain and contributing to impairment of synaptic plasticity and neurogenesis. A link exists between obesity and the extrinsic apoptotic pathway, as systemic inflammation is known to induce TNF secretion leading to binding to the TNF receptor (TNFR1) and subsequent activation of pro-apoptotic signaling cascades. Furthermore, increased levels of TNF block intracellular insulin signaling and this impact on insulin function may contribute to increased Aβ deposition (Alford et al., 2018). AD patients have been shown to have reduced insulin signaling in the CNS as evidenced by a lower CSF to peripheral insulin ratio as compared to healthy subjects (Alford et al., 2018). Different diets and nutritional intakes have been shown to impact cerebral Aβ deposition, as well as the gut microbiome composition (Pistollato et al., 2016). However, the mechanisms underlying the association of diet, the gut microbiome, amyloid formation, and enteric/peripheral neurons are poorly understood.

The enteric nervous system (ENS) is the largest component of the peripheral nervous system (PNS) and is capable of functioning independently of the CNS. The ENS functions include high volume communication with the CNS via the vagus nerve. Enteric neurons are known to express APP, and APP and Aβ levels were found to be increased in the intestinal epithelial cells of mice fed a diet high in saturated fat and cholesterol (Puig et al., 2013). Additionally, one study found amyloid plaques in the intestines of AD patients (however, a caveat of the study was that plaques were only found in 2 patients) (Joachim et al., 1989). However, when taken overall the limited

studies involving the role of the ENS in AD pathology show conflicting results highlighting this gap in understanding.

Animal models are a valuable way of studying AD, especially when assessing the genetic background of the disease and its association with obesity. Specifically, AD transgenic rodents provide a relatively cost- and time-effective method for studying genetic mutations associated with AD (LaFerla and Green, 2012). Transgenic mice that overproduce mutant APP (e.g. 5xFAD) show AD pathology similar to that in the human brain. Additionally, Aβ deposition into senile plaques is age-dependent in these mice with plaques forming in early adulthood (4 – 6 months) paralleling the age-dependent progression of AD pathology in humans. The structure of the plaques that develop in transgenic mice brains is similar to the senile plaques in humans and consists primarily of a high concentration Aβ42 core and some Aβ40 (LaFerla and Green, 2012). Furthermore, a transgenic rat model (TgF344-AD) with two AD implicated mutations (one in both the APP and presenilin 1 genes) displays AD pathology in the brain including amyloid plaques and neuronal loss, while also showing Tau pathology (Saré et al., 2020). These rodent models are a useful tool for translational research on AD.

We aim to assess how the genetic background for AD, aging and environmental factors such as a high-fat, high-carbohydrate diet (HFHC) contribute to intestinal amyloidosis and intestinal neuronal death. For this, we investigated the impact of a HFHC on soluble amyloid beta abundance and enteric neurodegeneration in the 5xFAD mouse model, and subsequently the soluble amyloid deposition in the colons of the TgF344-AD rat model in two different time points. These investigations will shed light on how GI and CNS disease mechanisms impact AD, which can in turn lead to the development of better treatments for this neurodegenerative disease.

Methods

Animals

5xFAD (*n* = 20) and WT (*n* = 12) female mice were born at Emory University Animal Facility and at 8 weeks of age were group housed by sex, and mixed genotype (22–23 °C with a 12/12-h light-dark cycle). Mice were used for behavioral assays and assessment of colon tissue. Female and male TgF344-AD (*n* = 15) and WT (*n* = 16) rats bred in the Weinshenker Lab were used in this study for the assessment of colon tissue. Rats were kept group housed at the Emory University Animal Facility in a colony room (22–23 °C with a 12/12-h light-dark cycle) and were humanely euthanized at either 6 or 15 months of age to allow for the evaluation of impact of aging in the intestinal outcomes.

Diet intervention

Mice received drinking water and standard chow diet (4% fat diet 7001, Envigo) or high-fat high-carbohydrate diet (HFHC) (42% kcal from fat, TD.88137, Envigo) plus 30% (w/v) fructose solution (F012, Sigma-Aldrich) available ad libitum at 2 months of age for 8 weeks. TgF344-AD rats received regular chow diet available ad libitum and regular drinking water until they were euthanized.

Behavior test

Behavioral tests were conducted on both HFHC and control diet (CD) fed 5xFAD mice and CD fed WT mice to track fecal matter transit through the colon. Rate of fecal output (pellets produced per 5 minutes, over 30-minute observation periods) were measured, and fecal weight and water content were assessed.

Fecal output assay protocol

Prior to assay, 1 liter (~12 cm x 25 cm) translucent beakers were sterilized and covered with aluminum foil. On the day of assay, each mouse was placed into an individual beaker in the testing room. Then each beaker was covered with a paper towel and a timer was set for 30 minutes. In 5-minute intervals, the number of fecal pellets in each beaker was counted and recorded. For consistency, only the cumulative number of pellets observed was recorded. After conclusion of the session, mice were returned to their home cages and fecal pellets were collected for the fecal water content assay.

Fecal water content assay protocol

Individual 1.5 sterilized microcentrifuge tubes were weighed for each mouse and the empty weights of the tubes were recorded. Fresh fecal pellets were collected into tubes in conjunction with fecal output assay, and the weights of the full tubes were recorded. Tubes were then placed on a heat block set to 90-100 degrees Celsius under a fume hood (to increase air flow and prevent odor). Pellets were incubated for 24 hours to remove water. After incubation, the weights of the tubes with dried pellets were recorded. To calculate percent water content, the following equations were used:

Percent water content calculation:

Full tube weight – Empty tube weight = Wet weight of fecal pellets

Dry tube weight – Empty tube weight = Dry weight of fecal pellets

(Wet weight – Dry weight) / Wet weight = Water content ratio

Water content ratio x 100 = Percent water content

Tissue collection and samples preparation

Female 5xFAD mice were sacrificed in the early morning at the end of their active time/transition to the start of their inactive time under isoflurane anesthetic. Colon tissue was harvested, flushed with phosphate buffered saline (PBS) and stored at – 80 degrees Celsius. Female and male TgF344-AD rats were euthanized in the Weinshenker Lab at 6 and 15 months of age and the proximal colon tissue was sent to the Sampson Lab for analysis.

Colon samples were assessed using immunoblotting and immunoassays. Briefly, protein was isolated from the proximal colon samples using a 1X homogenization buffer (1% Triton-X 100, 1.0M Tris HCL, 0.5M MgCl2, 0.1M EDTA, pH 7.2, plus protease inhibitor (Roche). The samples were homogenized using a sonicator at 20 Hz for approximately ninety seconds. After sonication, samples were centrifuged at 13,000 rpm (4 degrees Celsius) for ten minutes. The supernatant was collected for protein quantification. The protein concentration of supernatant was assessed using protein assay/ BCA (Pierce Scientific #23225). After BCA analysis, 5 µl of each sample was aliquoted into individual tubes with 5 µl of β-mercaptoethanol (BME) and tubes were heated at 95 degrees Celsius for five minutes. Samples (5 µl each) were pipetted into western blot tris-glycine 4-20% polyacrylamide gel wells and the gels were run at 150 V for one hour. After the running phase, the gels were transferred to an Immobilon P transfer membrane (#IPVH00010) with pore size 0.45 µm and run at 10 V overnight at 4 degrees Celsius. The following day, the membranes were blocked in 5% bovine serum albumin (BSA) in tris-buffered saline with tween (TBST) for one hour and then the membranes were probed overnight at 4 degrees with the primary antibodies (1:1000): Protein Gene Product 9.5 (PGP9.5; a universal marker of enteric and peripheral neurons), choline acetyltransferase (ChAT; a neuronal marker involved in the synthesis of acetylcholine and the primary excitatory neuron in the ENS), Nitric

Oxide Synthase (nNOS; an enzyme that catalyzes the formation of nitric oxide and marks the primary inhibitory neuron in the ENS), or anti-β-actin (antibody-loading control). Additionally, the membranes were probed with neuroepithelial stem cell protein (Nestin) and SRY-Box Transcription Factor 2 (SOX2); two markers of intestinal neurogenesis. Then, the membranes were exposed to species-appropriate horse- radish peroxidase (HRP)-conjugated secondary antibody (1:1000) for one hour. Bands were visualized by chemiluminescence, and protein band optical intensity was measured using densitometric analysis (FIJI software). Values were normalized relative to β-actin levels from the same sample.

Multiplexed immunoassays

Intestinal Aβ abundance in the Tg-F344 rat and 5xFAD mouse models (vs. respective WT controls) was evaluated using rodent-specific multiplex immunoassays (V-PLEX Aβ Peptide Panel 1 (6E10) Kit for rats and V-PLEX Aβ Peptide Panel 1 (4G8) Kit for mice). Inflammatory cytokine concentrations in male and female TgF344-AD and WT rats at 6 and 15 months of age was assessed with a multiplex immunoassay (V-PLEX Proinflammatory Panel 1 Rat Kit K15059D-1). Inflammatory cytokine concentrations in female 5xFAD and WT mice was assessed using a multiplex immunoassay (V-PLEX Proinflammatory Panel 1 Mouse Kit K15048D-1). For protein concentrations for the immunoassays, 1 ug/uL was used.

Statistical analyses

For statistical comparisons between the groups, unpaired t test and two-way ANOVA followed by Tukey's multiple comparisons test were used where applicable. Data are represented as the

mean ± standard error of the mean (SEM). For all analyses, 0.05 P value threshold was considered statistically significant. Analyses were performed using GraphPad Prism 9.

Results

5xFAD mice do not present intestinal soluble amyloid

Aβ deposition into insoluble plaques is a hallmark of AD in the brain reported in the 5xFAD mouse model. We predicted that this pathology would be mirrored in the colon of 5xFAD mice, as indicated by increased soluble amyloid, the precursor to insoluble plaque formation. No Aβ difference was observed between 4-month-old 5xFAD female mice and 4-month-old WT littermates. No detectable Aβ concentrations were found in either genotype group (data not shown) when assessed via immunoassay with a V-PLEX Aβ Peptide Panel 1 (4G8) Kit for mice, suggesting that the mutant APP gene and/or PSEN1 gene does not become processed in the murine colon as it is in the brain (possibly due to inhibition of gene expression or protein function). To determine if this is common in other rodent models, we next sought to address a potential genotype-dependent effect on amyloid pathology in the TgF344-AD rats.

TgF344-AD rats present intestinal soluble amyloid

The TgF344-AD rat model is also known to present amyloid plaques in the brain, but intestinal pathology is currently unknown. Since our observations in 5xFAD mice demonstrated a lack of Aβ in the murine intestine, we sought to determine whether Aβ could be observed in the TgF344-AD rat colon. TgF344-AD rats expressed significantly increased Aβ compared to WT littermates (**Figure 1**). Increased Aβ40 ($P < 0.0001$) (**1.a**) and Aβ42 ($P < 0.0001$) (**1.b**) were

observed in the TgF344-AD rats. No changes were observed in intestinal amyloid concentrations of Aβ40 (**2.a**) or Aβ42 (**2.b**) over time between the 6-month and 15-month age groups (**Figure 2**). These results support a mechanism of increased cleavage of APP into Aβ products in the intestine of these transgenic rats.

TgF344-AD 15-month-old rats present sex-dependent intestinal differences in soluble amyloid

AD predominately affects women and two-thirds of patients are female (Alzheimer's Association, 2021). Thus, we wanted to know if the sex-biased incidence rate of the disease could be supported by potential sex differences in presentation of TgF344-AD rat amyloid pathology in the colon. Female 15-month-old TgF344-AD rats expressed significantly increased Aβ compared to 15-month-old male TgF344-AD rats (**Figure 3**). Increased Aβ40 ($P = 0.017$) (**3.a**) and Aβ42 ($P = 0.0045$) (**3.b**) were observed in the females. No change in intestinal amyloid concentrations of Aβ40 or Aβ42 was observed between sexes in the 6-month-old group. These results suggest that intestinal Aβ pathology is more pronounced in females in an age dependent manner. Having identified evidence of Aβ pathology in the colon of transgenic rats, we next wanted to assess if the loss of hippocampal and cortex neurons reported in these rats was paralleled in the intestinal neurons of these rats.

No genotype effect on neuronal marker expression in TgF344-AD 15-month-old rats

Based on the finding that the TgF344-AD rat model produces intestinal Aβ and the literature suggesting that Aβ pathology drives neuronal apoptosis in the cortex and hippocampus, we were interested in assessing expression of key enteric neuronal markers in the colon to determine if the

approximately 40% loss of hippocampal and cortex neurons by 16 months identified in the TgF344-AD rat model was mirrored in the ENS. No decreases were found in intestinal concentrations of PGP9.5 (**4.a**), ChAT (**4.b**), or nNOS (**4.c**), providing support for no abnormal neuron loss in the intestine. Stemming from the understanding that neuroinflammation can increase the risk of AD and be accelerated by amyloidosis, our next step was to assess inflammation of the colon in the TgF344-AD rats.

Interferon-gamma (IFN-γ) concentration is age-dependent in TgF344-AD rats

We next wanted to know if the CNS inflammatory response linked to AD was mirrored in the intestine. We hypothesized that due to increased abeta we would observe increased presence of proinflammatory markers in the colon of the transgenic rats signaling an enhanced inflammatory response. We analyzed 9 inflammatory cytokines (IFN-γ, IL-1β, IL-4, IL-5, IL-6, IL-10, IL-13, CXCL1, TNF-α) in the colon of the TgF344-AD rats. After assessing the data for genotype and aging effects, only the concentration of IFN-γ was significantly different between 15-month-old and 6-month-old TgF344-AD rats ($P = 0.019$) (**Figure 5**). Thus, no genotype-dependent difference was observed in IFN-γ expression.

HFHC diet decreases fecal percent water content in 5xFAD mice

High fat, high carbohydrate (HFHC) diets are known to induce intestinal, systemic, and neuro-inflammation. Based on current understanding of an interaction between HFHC diets and AD-linked genetic backgrounds to promote pathology, we predicted that HFHC treatment of 5xFAD mice would exacerbate intestinal pathology. We first sought to determine whether hallmark constipation features (reduced fecal water content and fecal pellet production) were affected in

these mice. Female 5xFAD mice fed a HFHC diet had significantly less water composition of their fecal pellets (P = 0.014) compared to CD fed 5xFAD females (**6.a**). CD fed WT mice were used as controls. Pellets produced over time did not significantly differ between groups (**6.b**). These results suggest that HFHC diet alters intestinal water absorption, as a measure of constipation, in the 5xFAD mouse model.

HFHC diet regulates inflammatory cytokines in 5xFAD mice

Based on the understanding that a HFHC diet induces inflammation, we expected to see upregulation of proinflammatory cytokines in the colon of HFHC diet fed 5xFAD mice, thus confirming an increase in intestinal inflammatory response. Female 5xFAD mice fed a HFHC diet expressed significantly increased IL-1β (P = 0.03) (**7.a**) and CXCL1 (P = 0.004) (**7.b**) and significantly decreased IL-5 (P = 0.0005) (**7.c**) compared to CD fed 5xFAD females (**Figure 7**; Rodrigues et al., unpublished). There were no changes in the other assessed inflammatory markers: IFN-γ, TNF-α, IL-2, IL-6, and IL-10 (data not shown). These results are in accordance with our predictions as concentrations of two proinflammatory markers (IL-1β and CXCL1) increased in response to the HFHC diet.

HFHC diet fed 5xFAD mice do not present intestinal soluble amyloid

Since intestinal water absorption could be modulated by amyloid and inflammation in the ENS, we next wanted to understand how/if a HFHC diet impacts intestinal amyloid concentrations. No Aβ difference was observed between HFHC diet fed 4-month-old 5xFAD female mice and CD fed 4-month-old 5xFAD female mice. No detectable Aβ concentrations were found in either diet group (data not shown). A prior assay showed a lack of detectable intestinal soluble amyloid in

both WT and transgenic mice, and these results suggest that a HFHC diet alone does not increase presence of soluble Aβ in the colon.

HFHC diet does not impact intestinal neuronal marker expression in 5xFAD mice

Neuroinflammation associated with AD has been implicated in increased death of neurons in the brain (Kinney et al., 2018). We wished to investigate if HFHC-induced inflammation affected neuronal outcomes in the ENS similarly. HFHC diet did not affect colon concentrations of key neuronal and neurogenesis markers (**Figure 8**). No significant differences were observed in concentrations of nNOS (**8.a**), ChAT (**8.b**), PGP9.5 (**8.c**), Nestin (**8.d**), or SOX2 (**8.e**) between HFHC and CD fed 4-month-old 5xFAD female mice.

Discussion

A genotype effect exists on intestinal AD pathology

Elevated concentrations of soluble Aβ have been implicated in the occurrence and progression of AD pathology in the brain and CNS by precipitation of increased formation of senile plaques (Kumfor et al., 2017). Although amyloidosis as a main component of AD pathology in the CNS is well established (Kumfor et al., 2017; Haass and Selkoe, 1993), relatively little research exists on amyloid deposition in the GI tract in the context of AD. Our findings indicate that genotype impacts intestinal soluble amyloid in the TgF344-AD rat model of AD but not in the 5xFAD mice. These findings suggest that the variation in the specific AD-implicated mutations in the human APP and PSEN1 transgenes between the two rodent models may contribute to the detectable presence, or lack thereof, of intestinal soluble Aβ. Thus, the AD-linked mutations

specific to the TgF344-AD rats contribute to elevated soluble Aβ concentrations in the colon. Transgene expression is driven by a distinct promoter in each of these rodent models (Saré et al., 2020; LaFerla and Green, 2012) and this difference may contribute to variations in gene expression (e.g. increased APP expression in TgF344-AD rats). Alternatively, it is possible that the TgF344-AD rat model has more of the β- and γ-secretase enzymes involved in APP cleavage present in the intestine than the 5xFAD mouse model. Interestingly, TgF344-AD rats display increased IFN-γ in the colon at 15 months as compared to 6 months. Our data demonstrates that this increase in IFN-γ appears at a late age during which these rats are established to simultaneously present an increase in hippocampal and cortex plaque deposition, and loss of approximately forty percent of their hippocampal and cortex neurons. As Aβ deposition is known to precipitate an acute immune response in the CNS contributing to increased production of proinflammatory cytokines including IFN-γ, it is possible that Aβ deposition in the colon could promote an increase in IFN-γ. While we cannot confirm Aβ promoting IFN-γ in the colon without first assessing for presence of insoluble Aβ in the intestine, our data do demonstrate the presence of processed Aβ in the colon with the capacity to form aggregates.

No genotype effect or HFHC diet impact on intestinal neuronal marker expression

Our data show that expression of several neuronal markers in the colon providing critical roles in the ENS do not change based on the TgF344-AD rat genotype or introduction of a HFHC diet in the 5xFAD mice. These findings suggest that enteric neuron loss is likely not a major component of AD pathology in the intestine, in these models. However, imaging and intestinal stereology to determine potential change in neuron counts would be an important next step to confirm our results.

HFHC diet as an environmental factor impacts intestinal inflammatory response

Neuroinflammation is a major hallmark of AD pathology in the CNS and enhanced peripheral inflammatory marker production resulting from external factors such as a HFHC diet can contribute to the CNS inflammatory response via increased cytokine movement from the intestine to the brain (Kinney et al., 2018). Our findings provide support for this mechanism based on elevated cytokine production in the ENS of HFHC diet fed 5xFAD mice, and indicate that the regulation of the local enteric inflammatory response is directly impacted by a HFHC diet, as evidenced by upregulation of pro-inflammatory (IL-1β, CXCL-1) and downregulation of anti-inflammatory (IL-5) markers in the colon. The implications of intestinal inflammation on the brain include insulin impairment, upregulation of processes contributing to neuroinflammation, and an overall increase in risk for onset of AD (Kinney et al., 2018).

HFHC diet impacts GI motility

A genetic link exists between AD and some GI tract disorders (Adewuyi et al., 2022). A group of genes have been associated with having both AD and GI disorders including gastroesophageal reflux disease, peptic ulcer disease, and irritable bowel syndrome (Adewuyi et al., 2022). Constipation is a symptom of many GI disorders and defined by lower-than-normal fecal percent water content. Constipation is an indicator of slower motility of feces through the large intestine where water absorption takes place. Our findings suggest that HFHC diet can contribute to constipation. Constipation can be associated with GI tract inflammation, and peripheral inflammation is linked with increased risk for occurrence and progression of AD (Nakase et al., 2022).

Conclusions

Our results suggest that a genotype effect exists contributing to AD pathology in TgF344-AD rats. The AD genotype elevates intestinal soluble amyloid compared to wild-type rats, and female transgenic rats display a higher level of soluble Aβ compared to male transgenic rats. In addition to intensifying the local inflammatory response in the periphery, increased intestinal Aβ production could compound the neuroinflammatory response in the brain via increased production and translocation of proinflammatory cytokines across the BBB. Additionally, the increase in soluble amyloid could lead to insoluble plaque build-up in the intestine which could potentially contribute to gut barrier dysfunction and reduced intestinal motility. A reasonable next step would be to perform an immunoassay for insoluble Aβ in the colon of the TgF344-AD rat model to determine if these rats present plaque formation as part of their intestinal AD pathology.

Our mouse study results suggest that a HFHC diet may modulate inflammation of the intestine, but in a manner that appears to be independent of Abeta production. Peripheral inflammation is a known risk factor for AD pathology both in the CNS and GI tract. Furthermore, HFHC diet contributes to onset of symptoms of GI disorders known to be associated with AD. These findings are an important step to improving our understanding of intestinal AD pathology, how it contributes to the hallmarks of AD pathology in the brain, and the evolving characterization of the disease.

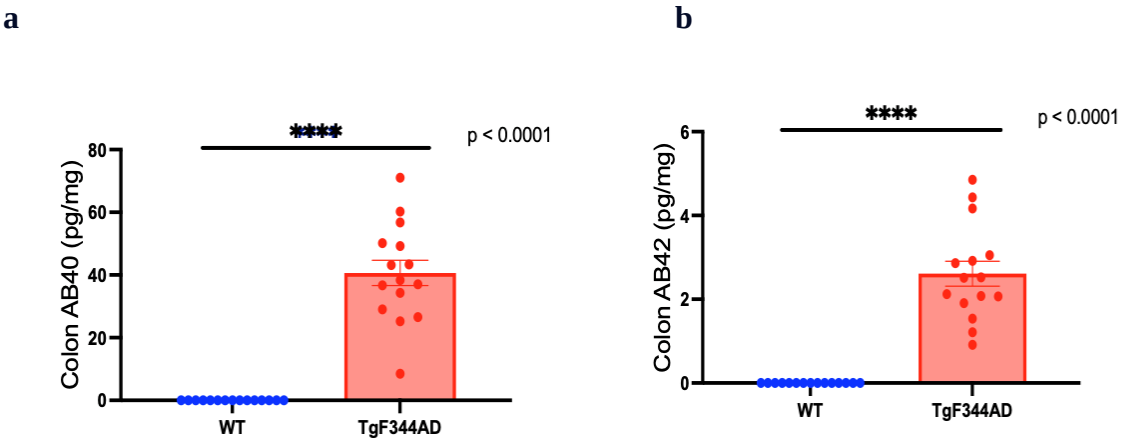


Figure 1: Presence of soluble Aβ40 (**a**) and Aβ42 (**b**) in the colon of TgF344-AD rats

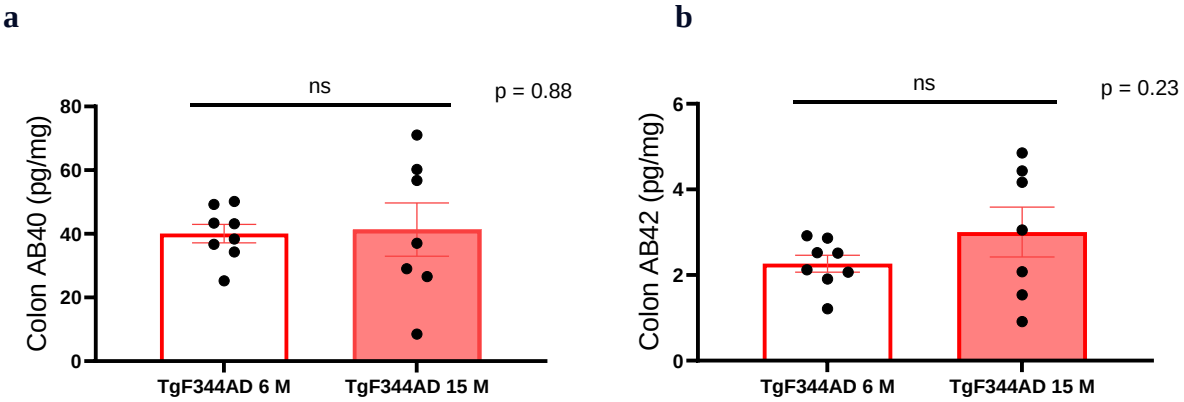


Figure 2: No change in soluble Aβ40 (**a**) or Aβ42 (**b**) over time in the colon of TgF344-AD rats

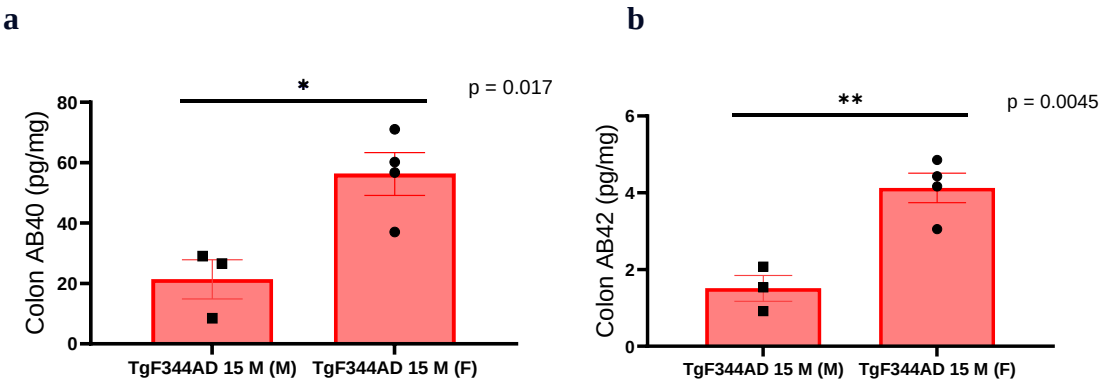


Figure 3: Sex difference in soluble Aβ40 (**a**) and Aβ42 (**b**) in the colon of 15-month-old TgF344-AD rats

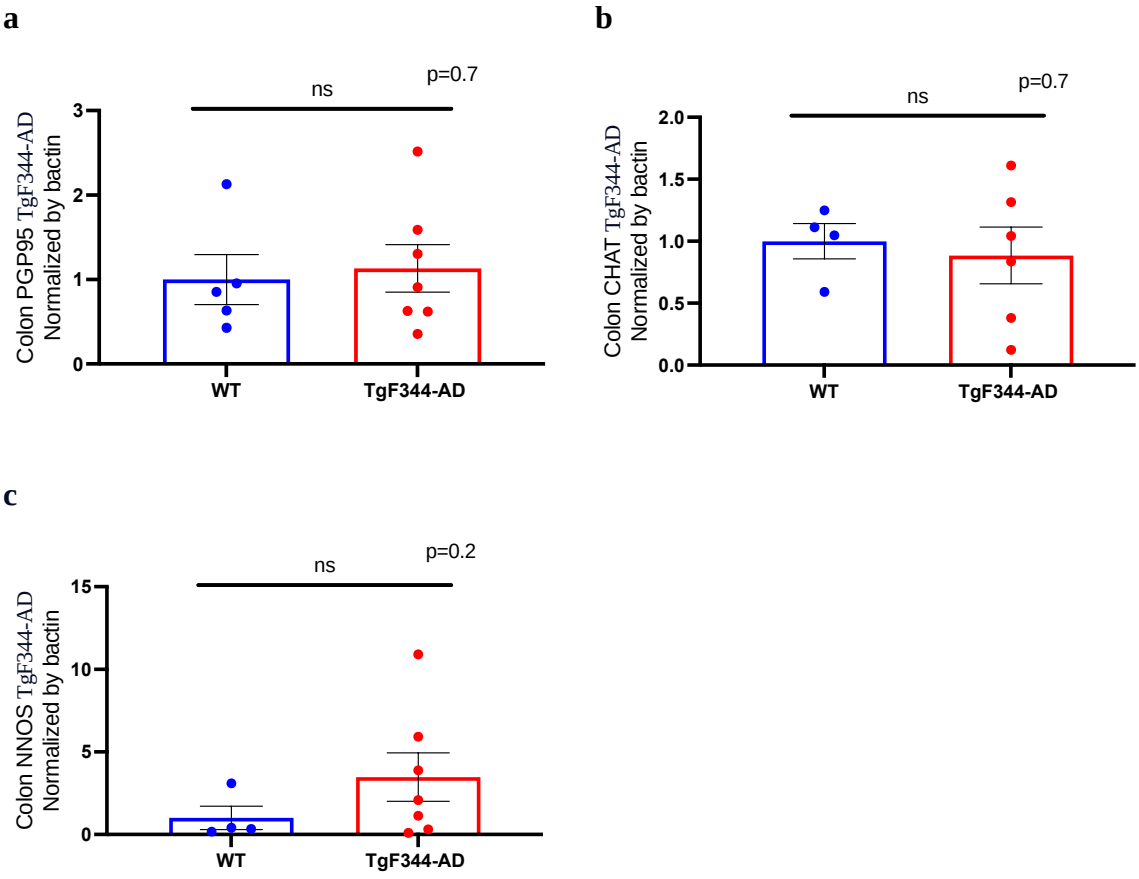


Figure 4: No genotype-dependent change in expression of neuronal markers: PGP9.5 (**a**), ChAT (**b**), and nNOS (**c**) in the colon of 15-month-old TgF344-AD rats

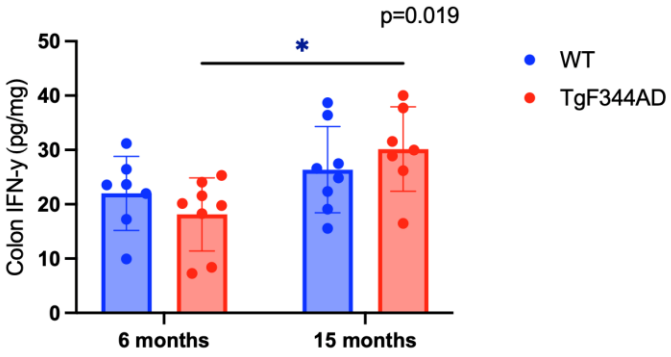
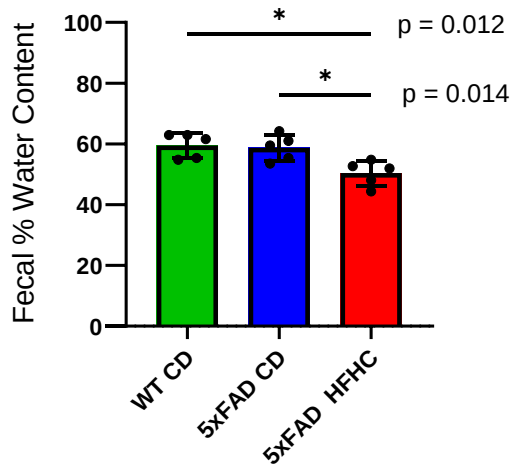


Figure 5: Age impact on IFN-γ concentration in the colon of WT and TgF344-AD rats

a



b

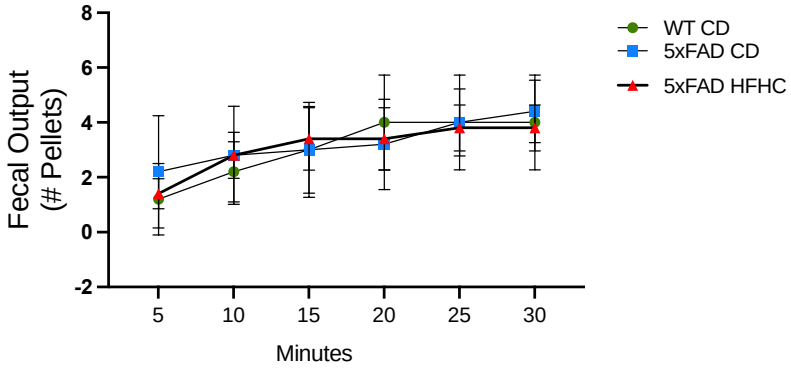


Figure 6: HFHC diet impact on fecal percent water content (**a**) and fecal output (**b**) in 5xFAD mice

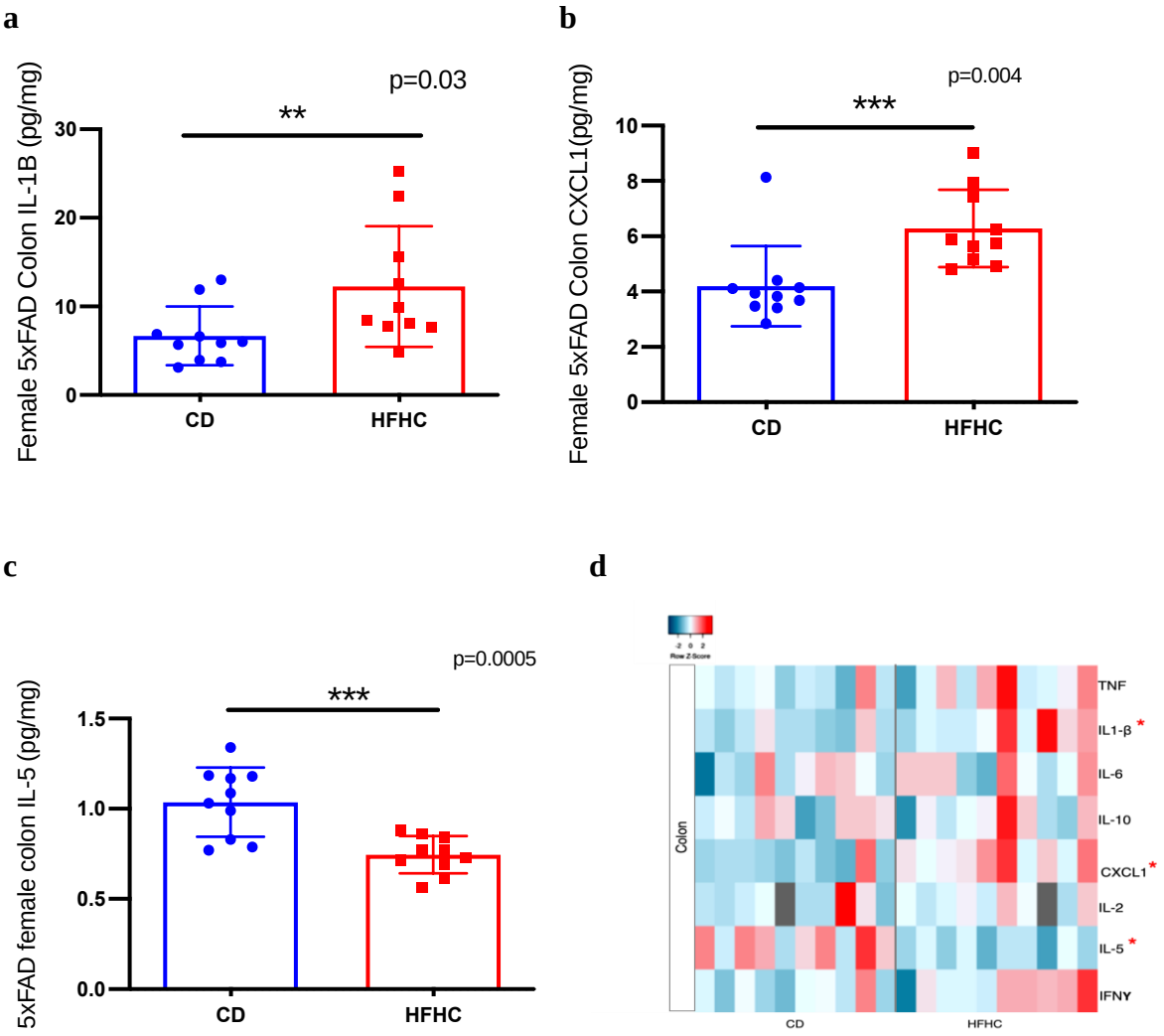


Figure 7: HFHC diet impact on IL-Iβ (**a**), CXCL1 (**b**), and IL-5 (**c**) in the colon of 5xFAD female mice; heatmap of assessed inflammatory markers in HFHC vs CD fed 5xFAD mice (**d**)

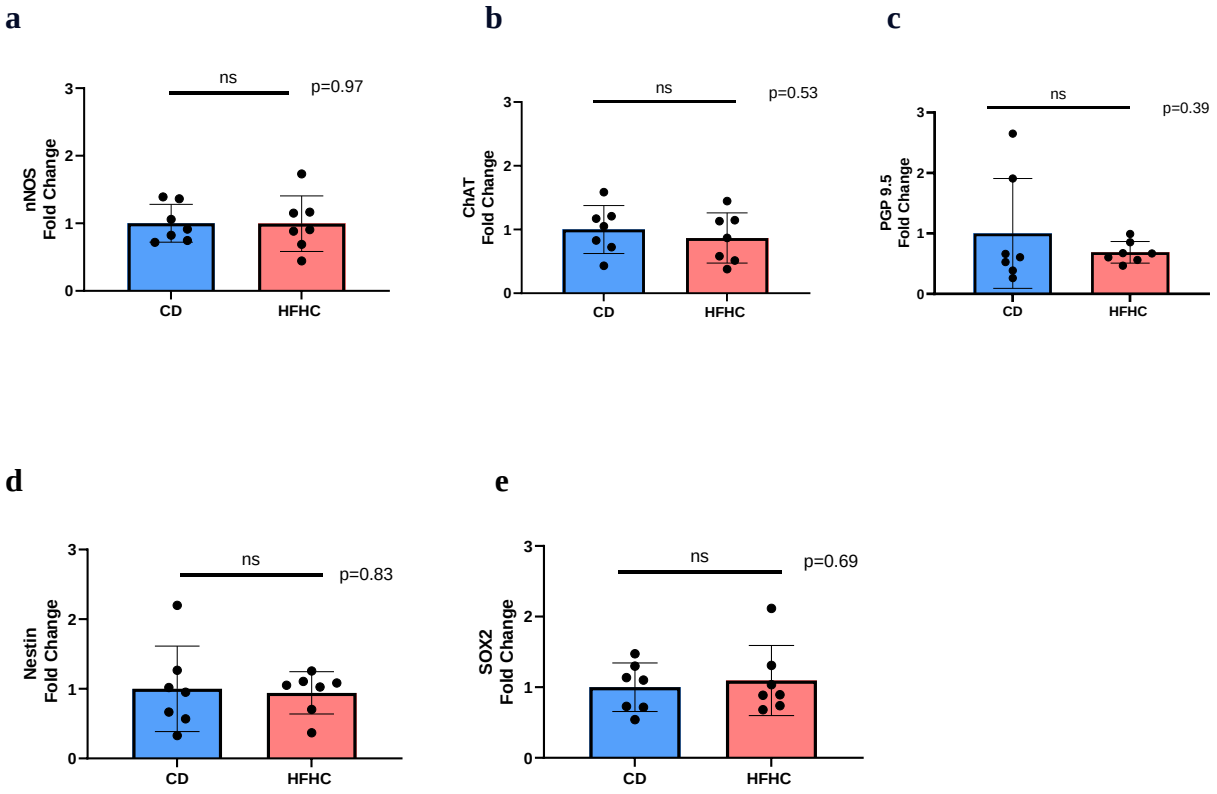


Figure 8: No change in nNOS (**a**), ChAT (**b**), PGP9.5 (**c**), Nestin (**d**), and SOX2 (**e**) in the colon of HFHC vs CD fed 5xFAD mice

References

2021 Alzheimer's disease facts and figures. Alzheimers Dement. 2021;17(3):327-406.

Adewuyi EO, et al. A large-scale genome-wide cross-trait analysis reveals shared genetic architecture between Alzheimer's disease and gastrointestinal tract disorders. Commun Biol. 2022;5:691.

Alford S, et al. Obesity as a risk factor for Alzheimer's disease: weighing the evidence. Obes Rev. 2018;19(2):269-280.

Blasko I, et al. Costimulatory effects of interferon-gamma and interleukin-1beta or tumor necrosis factor alpha on the synthesis of Abeta1-40 and Abeta1-42 by human astrocytes. Neurobiol Dis. 2000;(6 Pt B):682-9.

Dang S, et al. Cleavage of amyloid precursor protein by an archaeal presenilin homologue PSH. Proc Natl Acad Sci U S A. 2015;112(11):3344-9.

De Strooper B, et al. Presenilins and γ-secretase: structure, function, and role in Alzheimer Disease. Cold Spring Harb Perspect Med. 2012;2(1):a006304.

Fowler DM, et al. Functional amyloid--from bacteria to humans. Trends Biochem Sci. 2007;32(5):217-24.

Greenwald J and Riek R. Biology of amyloid: structure, function, and regulation. Structure. 2010;18(10):1244-60.

Haass C and Selkoe DJ. Cellular processing of beta-amyloid precursor protein and the genesis of amyloid beta-peptide. Cell. 1993;75(6):1039-42.

Hengartner MO. The biochemistry of apoptosis. Nature. 2000;407(6805):770-6.

Joachim C, et al. Amyloid β-protein deposition in tissues other than brain in Alzheimer's disease. Nature.1989;341:226–230.

Kinney JW, et al. Inflammation as a central mechanism in Alzheimer's disease. Alzheimers Dement (N Y). 2018;4:575-590.

Kumfor F, et al. Clinical Aspects of Alzheimer's Disease. Adv Neurobiol. 2017;15:31-53.

LaFerla FM and Green KN. Animal models of Alzheimer disease. Cold Spring Harb Perspect Med. 2012;2(11):a006320.

Leong YQ, et al. Mechanisms of action of amyloid-beta and its precursor protein in neuronal cell death. Metab Brain Dis. 2020;35(1):11-30.

Matthews KA, et al. Racial and ethnic estimates of Alzheimer's disease and related dementias in the United States (2015-2060) in adults aged ≥65 years. Alzheimers Dement. 2019;15(1):17-24.

McGirr S, et al. Alzheimer’s Disease: A Brief Review. Journal of Experimental Neurology. 2020;1(3): 89-98.

Meraz-Ríos MA, et al. Inflammatory process in Alzheimer's Disease. Front Integr Neurosci. 2013;13(7):59.

Nakase T, et al. Impact of constipation on progression of Alzheimer’s disease: a retrospective study. Neurosci Ther. 2022;28(12):1964-1973.

Pistollato F, et al. Role of gut microbiota and nutrients in amyloid formation and pathogenesis of Alzheimer disease. Nutr Rev. 2016;74(10):624-34.

Puig KL and Combs CK. Expression and function of APP and its metabolites outside the central nervous system. Exp Gerontol. 2013;48(7):608-11.

Santiago JA and Potashkin JA. The Impact of Disease Comorbidities in Alzheimer's Disease. Front Aging Neurosci. 2021;13:631770.

Saré RM, et al. Behavioral Phenotype in the TgF344-AD Rat Model of Alzheimer's Disease. Front Neurosci. 2020;14:601.