2022

The Effect of Early Dietary Intervention on Alzheimer's Disease-Related Pathology and Cognitive Function in Mice

Anna Amelianchik

The Effect of Early Dietary Intervention on Alzheimer's Disease-Related Pathology and Cognitive Function in Mice

A Thesis Presented to the Faculty of
The Rockefeller University
in Partial Fulfillment of the Requirements for
the degree of Doctor of Philosophy

by
Anna Amelianchik
June 2022

The Protective Effect of Dietary Fats on Alzheimer's Disease-Related Pathology and Cognitive Function in Mice

Anna Amelianchik, Ph.D.
The Rockefeller University 2022

Alzheimer's disease (AD) is a fatal cognitive disorder with proteinaceous brain deposits, neuroinflammation, cerebrovascular dysfunction, and extensive neuronal loss. AD is a multifactorial disease, and lifestyle factors, including diet, are likely associated with the development of AD pathology. Since obesity and diabetes are recognized as risk factors for AD, it might be predicted that a high fat diet (HFD) would worsen AD pathology. However, modeling HFD-induced obesity in animal models of AD has yielded inconclusive results. Some studies report a deleterious effect of HFD on Aβ accumulation, neuroinflammation, and cognitive function, while others report that HFD worsens memory without affecting AD brain pathology. Moreover, several studies report no major effect of HFD on AD-related phenotypes in mice, while other studies show that HFD might, in fact, be protective. The lack of a clear association between HFD consumption and AD-related pathology and cognitive function in AD mouse models might be explained by experimental variations, including AD mouse model, sex of the animals, composition of the HFD, and timeline of HFD consumption. Our study examined the effect of varying the timeline of HFD or control diet (CON) consumption on AD-related pathology and cognitive function in transgenic Tg6799 AD mice. HFD consumption that started at or before 3 months-of-age, prior to severe AD pathology, had protective effects in AD mice. Specifically, it reduced extracellular beta-amyloid (Aβ) deposition, decreased fibrinogen extravasation from blood vessels into the brain parenchyma, and improved cognitive function. RNAseq analysis revealed that HFD affected the expression of genes in the AD mouse cortex related to the stress response, protein folding, endoplasmic reticulum stress, chaperone-mediated protein folding, and the immune system process in AD mice. However, delaying HFD consumption until 6 months-of-age, when AD pathology is ubiquitous, did not provide neuroprotection in AD mice, as there was no change in cortical Aβ deposition in 11-month-old mice. Surprisingly, despite the delayed onset of HFD consumption, HFD still reduced the extravasation of fibrinogen into the brain. Overall, we demonstrate that the timeline of HFD consumption plays an important role in how dietary fats affect AD-related pathology and cognitive function in a transgenic mouse model of AD.

Эта научно-исследовательская работа посвящается моим родителям, Гульсине и Валерию Амельянчик, с любовью. Ну вот уже и полно.

Acknowledgements

I thank the Strickland Laboratory for fruitful discussion of experimental results included in this thesis over many Alzheimer's Disease Meetings, Lab Meetings, and casual conversations at the bench; Drs. Jeffrey Friedman and Kristina Hedbacker for their assistance with EchoMRI experiments; Caroline Jiang and Neha Singh for their guidance on statistical analysis; The Rockefeller University Genomics Resource Center for its help with sample processing for the RNAseq experiment; Drs. Thomas Carroll and Matthew Paul for their help with RNAseq data analysis; Dr. Dorit Farfara for the invaluable help with tissue collection; and Dr. Pradeep Singh for his guidance in contact system experiments. I would also like to thank my Thesis Committee Members, Drs. Paul Cohen, Jan Breslow, and Li Gan for helpful discussion of results and experimental strategy as well as Dr. Matthew Flick for taking his time to evaluate this work and participate in its discussion. Finally, I would like to sincerely thank Drs. Sid Strickland and Erin Norris for their guidance and mentorship, friendship and support, as well as the trust and intellectual freedom that molded me into the scientist that I am today. This work was supported in part by The Rockefeller University Women & Science Initiative.

Table of contents

List of Figures

Chapter 1: Introduction

Over the past century, life expectancy in the United States has increased dramatically due to successful treatments for serious illnesses such as cardiovascular disease and cancer [1]. However, a longer life expectancy leads to a larger elderly population, which is at high risk of dementia [2]. Therefore, prophylactic and therapeutic approaches for dementia are in high demand. Alzheimer's disease (AD) is the most prevalent form of dementia, which affected more than 6 million people in the United States in 2021 [3]. This number is projected to rise to 13 million Americans by 2050. Despite the prevalence of AD, this disease is the only top 10 cause of death that cannot be prevented, cured, or even slowed [4]. Currently there are 6 FDA-approved pharmacological therapies for AD, but they are not curative and only offer a modest clinical benefit [5].

AD is a complex multifactorial disease [6], and its symptoms vary greatly among patients, but commonly include difficulty remembering recent events and names, impaired decision making, and behavioral changes [3]. Histopathological hallmarks of AD include extracellular deposits of beta-amyloid (Aβ) in the form of senile plaques and intracellular inclusions of hyperphosphorylated tau protein in the form of neurofibrillary tangles [3]. AD is also characterized by neuroinflammation, profound neuronal loss, and vascular abnormalities [3].

To identify novel therapeutic approaches for AD, we need a better understanding of genetic and environmental risk factors that contribute to the development of the disease. Although in most cases a genetic cause for AD cannot be identified, a very small subset of patients suffers from the familial form of AD where the disease is caused by a mutation in either amyloid precursor protein *(APP)* or presenilin 1 or 2 *(PS1, PS2)*. Familial AD accounts for less than 1% of cases, and it typically manifests itself in patients under 65 years-of-age [7]. Sporadic AD typically occurs in those who are over 65 years, and the largest risk factor is carrying the E4 allele of the apolipoprotein (*APOE)* gene [8]. Several lifestyle factors, also called modifiable factors, have been linked to the development of AD. For example, people with more years of formal education and/or a mentally stimulating job or those who stay socially engaged are less likely to develop AD [9, 10], although it is unclear how these factors contribute to a reduced risk of AD. Furthermore, history of a traumatic brain injury or poor cardiovascular health has been shown to increase AD risk [11, 12].

Among cardiovascular risk factors, hypertension and obesity have been shown to strongly associate with an increased risk for developing AD, particularly in midlife (defined as 40-59 years in most studies) [13-15]. A meta-analysis concluded that by 2050, AD prevalence will be 9% higher in the United States than previously projected due to the drastic increase in the population's obesity [16]. However, a systematic review of several large well-controlled studies reported that obesity beginning in late life (60+ years) was not associated with higher risk for developing AD [17]. This study also concluded that a low body mass index (BMI) in midlife significantly increased AD risk. These findings contributed to the so-called "obesity paradox". Additionally, in 2015, the largest retrospective study to date conducted on 2 million people age 40+, examined the association between BMI and dementia [18]. It showed that underweight people had the highest risk for dementia compared to individuals with a healthy weight in midlife, and overweight individuals had the lowest risk for developing AD in late life. Some authors argue that the inverse

association between BMI and dementia can be attributed to reverse causation—a phenomenon that makes higher BMI appear protective when BMI assessments are made less than 20 years before AD diagnosis, i.e. when BMI assessments may be biased by preclinical AD and weight loss associated with it [19]. Future studies are needed to investigate why patients with preclinical AD lose body weight and whether preventing weight loss in that population would prevent conversion to AD or slow AD progression. Additionally, future studies are needed to address if other determinants of obesity, such as visceral fat stores, might predict late-life health outcomes more accurately and facilitate the translation of animal studies to clinical practice.

In animal studies, calorie-dense diets, such as those high in fat, are often used to induce weight gain and determine the association between obesity and AD-related phenotypes. In mouse models of AD, HFDs that range between 32-60% fat [20, 21] have been shown to exacerbate AD-related pathology, such as Aβ plaque load and microglial cell activation, and impair cognitive function [22, 23]. However, another study has shown that consumption of a high fat diet (HFD) has no effect on Aβ or tau pathology in the brains of other AD transgenic mice, while it is still capable of accelerating cognitive decline [21]. Conversely, other reports show that a HFD improves blood-brain barrier (BBB) integrity and cognition with or without reducing Aβ plaque load in AD mice [24, 25]. Overall, there seems to be no consistent association between diet-induced obesity and AD-related pathophysiology in animal studies, which might be due to technical discrepancies, such as the transgenic AD mouse model used, specific components of the diet, or time course of HFD administration.

Deleterious Effects of Dietary Fat Consumption on AD Pathophysiology in Animal Models

AD Mouse Models with APP and PS1 Mutations:

APP/PS1 Mouse Line - The association between HFD consumption and AD has been studied in different AD mouse models, which replicate key pathologies documented in AD patients, including Aβ accumulation, tau hyperphosphorylation, neuroinflammation, and cognitive decline. Many mouse models of AD focus on one aspect of AD pathophysiology – the accumulation of Aβ in the brain. For example, APP/PS1 mice, which express human transgenes for APP with the Swedish mutation (K670N/M671L) and PS1 with the L166P mutation under the control of the neuronal-specific Thy1 promoter, are a widely used model of accelerated Aβ accumulation, which makes them a suitable model to study effects of long-term HFD consumption on AD pathology [26]. APP/PS1 mice begin accumulating Aβ in the cortex as early as at 6 weeks-of-age and in the hippocampus by 3-4 months [26]. By 7 months, APP/PS1 mice begin showing impaired learning and memory [27]. Walker et al. (2017) administered a HFD containing 60% fat or a low fat control diet (LFD) containing 10% fat to 2-month-old APP/PS1 mice and their wild-type (WT) littermates [28]. The mice remained on the diet until they were 12 months old and underwent behavioral testing at various ages. A subgroup of mice was fed a HFD until 9.5 months-of-age and then administered a LFD to determine whether HFD-induced effects could be reversed. In the two-trial Y maze, HFD impaired memory retention in 12-month-old APP/PS1 mice, and this effect was reversed in the APP/PS1 mice that switched to LFD. Nest building was also impaired by HFD in 9- and 12-month-old APP/PS1 mice as well as WT mice. Walker et al. (2017) also observed that HFD increased the levels of insoluble Aβ40 and soluble Aβ42 but not soluble Aβ40 or insoluble Aβ42 [28]. Interestingly, the increased production of soluble Aβ42 was not rescued in mice that

were part of the reverse trial, but insoluble Aβ40 and Aβ42 were reduced by the switch from HFD to LFD. Overall, Walker et al. (2017) demonstrated that long-term HFD consumption leads to cognitive perturbations and alters Aβ accumulation in APP/PS1 mice [28].

The effect of HFD on AD-related pathology and cognitive function in APP/PS1 mice was also examined by Bracko et al. (2020) who found that APP/PS1 mice fed a 42% kcal HFD starting at 4 months-of-age had no memory impairments in the Y maze or the object replacement test at 8- or 19 months-of-age, compared to APP/PS1 mice fed a normal chow [29]. However, HFD consumption impaired sensory-motor function in the balance beam test in APP/PS1 mice at 10- and 19-months-of-age. Bracko et al. (2020) also found that HFD administration increased the levels of soluble and insoluble brain Aβ40 in mid- and late-life, while the levels of soluble and insoluble Aβ42 were unaffected [29]. HFD also increased cortical Aβ plaque density in 21-, but not 11-month-old mice, while hippocampal plaque density was increased by HFD in both age groups. Notably, HFD increased microglia cell density in the hippocampus of both 11-month-old APP/PS1 and WT mice, but this effect was no longer significant at the later age. Bracko et al. (2020) also investigated the effect of HFD on cerebrovascular pathology [29]. HFD administration had no effect on the number of occluded capillaries in APP/PS1 mice, although APP/PS1 mice overall show an increased number of occluded capillaries compared to WT mice. Similarly, although cerebral blood flow is decreased in APP/PS1 mice compared to WT mice, HFD had no effect on blood flow velocity in either WT or APP/PS1 mice.

In sum, 42-60% HFD consumption affects the levels of Aβ subspecies and increases gliosis in APP/PS1 mice. It may also increase Aβ plaque deposition and induce cognitive deficits, but these changes likely depend on the time frame of HFD consumption, the composition of the control diet, the sensitivity of behavioral paradigms that assess cognition, and mouse age at the time of behavioral testing.

5XFAD Mouse Line - Recent studies have addressed the effect of HFD consumption on the 5XFAD mouse model, which overexpresses human APP with Swedish (K670N/M671L), Florida (I716V), and London (V717I) mutations as well as mutant human PS1 (M146L, L268V) under the control of the Thy-1 promoter [30]. This AD mouse line presents with rapid accumulation of Aβ and recapitulates major pathological and behavioral abnormalities observed in AD patients. 5XFAD mice are available on the original hybrid C57BL/6 x SJL background or on the congenic C57BL6 background. 5XFAD mice on the hybrid background begin accumulating extracellular Aβ at approximately 2 months-of-age and start showing cognitive deficits by 4 months [30]. 5XFAD mice on the congenic C57BL6 background also begin accumulating Aβ at approximately 2 months of age, but the age of onset of cognitive deficits in these mice is more varied at 3- to 6-months-age [31, 32]. Medrano-Jimenez et al. (2019) administered a 60% kcal HFD to 6-week-old 5XFAD mice and their C57BL/6 x SJL WT littermates for 8 months [33]. They found that HFD increases Aβ plaque deposition in the hippocampus without affecting learning and memory in the Morris water maze. Reilly et al. (2020) used same HFD in 5XFAD mice on the congenic C57BL6 background, but they delayed the administration of the diet until the mice were 3 months old [34]. After 2 months of HFD consumption, 5XFAD mice had significant alterations in whole body metabolism, such as reduced satiety, increased caloric intake, and increased energy expenditure, when compared to 5XFAD mice fed a low-fat control diet. HFD also increased the levels of circulating lipids and impaired glucose clearance. To investigate the effect of HFD on the

microbiome, the authors compared the bacterial composition of feces of 5XFAD and WT littermates. While HFD significantly changed microbiome composition in both WT and 5XFAD mice, there was no effect of genotype, as the mice showed similar taxonomical changes. An unbiased screen of transcriptomic changes showed that HFD consumption induced the expression of genes associated with the insulin signaling pathway, AD risk, including Apoe, and apoptosis. Finally, another group investigated the effect of dietary fats on AD-related pathology and cognitive function in aged 5XFAD mice. Lin et al. (2016) fed 13-month-old 5XFAD mice on the congenic C57BL6 background either a 60% kcal HFD or a LFD for 10 weeks [35]. Interestingly, HFD did not increase body weight in this 5XFAD mouse study, and food intake did not differ between HFD-fed WT and 5XFAD mice, indicating altered metabolism in these aged 5XFAD mice. Similarly, HFD failed to increase blood glucose levels or impair glucose clearance in 5XFAD mice. Despite the lack of physiological changes induced by HFD consumption, HFD-fed 5XFAD mice presented with worse cognitive function compared to 5XAD mice fed the control diet when tested in the Morris water maze. HFD-fed 5XFAD mice exhibited Aβ deposition around blood vessels of the brain, termed cerebral amyloid angiopathy (CAA), though parenchymal Aβ deposition was unchanged. HFD increased cortical and hippocampal superoxide levels. Additionally, Western blot analysis revealed increased expression of NADPH oxidase subunits and COX-2. This indicates that HFD consumption enhanced oxidative stress in 5XFAD mice. Overall, despite utilizing the same AD mouse model and the same HFD, these various studies did not arrive at the same conclusion regarding the effect of dietary fat consumption on AD-related pathology and cognitive function in 5XFAD mice, likely due to variable timelines of HFD administration.

In summary, in 5XFAD mice on the congenic C57BL6 background, 60% HFD has been reported to increase cerebrovascular Aβ deposition, impair learning and memory, enhance brain oxidative stress, and induce the expression of apoptotic, microglial, and amyloidogenic genes. However, it is difficult to draw parallels between the two studies described above due to the different ages of HFD consumption onset (3 vs 13 months), varied timelines of HFD consumption (10 weeks vs 3 months), and differences in the composition of the control diet (ingredient-matched control diet vs standard chow). Thus, additional studies are needed to elucidate the effect of HFD on AD-related pathology and cognitive function in this widely-used AD mouse model.

APP23 Mouse Line – Both 5XFAD and APP/PS1 mouse lines are considered aggressive models of AD since cortical Aβ plaque deposition starts as early as at 6 weeks-of-age [26, 30]. The APP23 line, however, exhibits AD-related pathology around 12 months, which may align with and be more relevant to the human condition. APP23 transgenic mice express human APP751 familial Swedish mutation (K670N/M671L) under the control of the mouse Thy-1 promoter [36]. APP23 mice begin accumulating Aβ in the brain at around 6 months-of-age [36]. However, cognitive decline in this AD mouse model precedes extracellular Aβ deposition and can be observed at 3 months-of-age [37]. Nam et al. (2017) administered a 40% kcal HFD to 1-year-old APP23 mice for 3 months and assessed spatial memory, Aβ pathology, and brain transcriptome and lipidome changes in ~15-month-old mice [38]. HFD consumption impaired learning and memory in the Morris water maze when compared to APP23 mice fed a normal diet with 16% kcal from fat. HFD also increased Aβ plaque deposition in the brains of APP23 mice. Interestingly, while HFD affected male and female APP23 mice similarly, female APP23 mice overall had more Aβ pathology. In line with these findings, HFD feeding increased TREM2 immunoreactivity in the

cortex of female HFD-fed APP23 mice, while there was no significant difference between APP23 mice fed either HFD or normal chow, suggesting that Aβ accumulation can modulate TREM2 protein expression in the brain. RNAseq revealed that HFD increased the expression of genes related to immune response and inflammation while it decreased the expression of genes related to neuronal projection and synaptic transmission. Finally, lipidomics analysis revealed that HFD increased the amount of various anionic phospholipids in APP23 mice that play an important role in phagocytosis and apoptosis.
Overall, administration of a 40% HFD later in life increases Aβ deposition and impairs cognitive function in APP23 mice, an AD mouse model with abundant Aβ deposition in "middle age". However, additional studies are needed to replicate these results, including studies that utilize an ingredient-matched control diet vs standard mouse chow to control for the sources of macronutrients, namely carbohydrate, protein, and fat, as well as added vitamins and minerals.

APP Knock-In Mouse Models:

$APP^{NL/NL}$ Mouse Line - Most AD models are transgenic mice that overexpress mutant human genes associated with AD. However, Saito et al. (2014) developed an AD mouse model that aimed to overcome potential artifacts introduced by non-physiological overexpression of APP or Aβ [39]. $APP^{NL/NL}$ knock-in mice express APP with a humanized Aβ region and the pathogenic Swedish "NL" mutation (KM670/671NL) under the control of the endogenous APP mouse promoter [39]. As a result, these mice express APP at wild-type levels in the appropriate cell types and brain regions, while also producing pathogenic Aβ40 and Aβ42 [39]. Interestingly, despite elevated production of mutant Aβ, $APP^{NL/NL}$ mice do not show plaque accumulation or cognitive impairment even at 18 months-of-age [40]. To investigate whether HFD triggers AD-related pathology and cognitive dysfunction in this mouse line, Salas et al. (2018) administered a 60% kcal HFD or a LFD to 2-month-old $APP^{NL/NL}$ mice [41]. $APP^{NL/NL}$ mice remained on the diet for either 4 or 16 months until they were 6- or 18-months-old, respectively. HFD increased body weight, induced hyperglycemia, impaired glucose clearance, and led to peripheral insulin resistance in 6-month-old $APP^{NL/NL}$ mice. Experimental diets did not induce increased or early soluble Aβ production or Aβ plaque deposition in the hippocampus when compared to $APP^{NL/NL}$ mice fed a control diet. BACE activity and tau phosphorylation were also unaffected by dietary fat consumption. Similarly, prolonged HFD consumption did not affect amyloidosis or tau phosphorylation in 18-month-old mice. Iba-1 and GFAP immunoreactivity were not affected by HFD consumption in either 6- or 18-month-old old $APP^{NL/NL}$ mice, indicating that HFD did not increase either microglia cell recruitment or astrogliosis in $APP^{NL/NL}$ mice. Behaviorally, neither 6- nor 18-month-old $APP^{NL/NL}$ mice fed a HFD presented with impaired contextual or cued fear memory. Salas et al. (2018) also used proton magnetic resonance spectroscopy (MRS), a noninvasive neuroimaging technique, to quantify brain metabolites associated with AD in live mice [41]. MRS showed that long-term HFD consumption decreased the ratio of N-acetyl aspartate (a marker of neuronal viability) to myo-inositol (a marker of gliosis). The authors also showed that long-term HFD consumption in $APP^{NL/NL}$ mice led to an impairment in long-term potentiation (LTP) but not long-term depression (LTD). In $APP^{NL/NL}$ mice, short- and long-term 60% HFD consumption failed to affect AD-related pathology or cognitive function, although HFD did include a decrease in hippocampal LTP. Therefore, by comparing the results obtained from HFD-fed $APP^{NL/NL}$ mice to those of other transgenic AD mouse lines, it is apparent that the other transgenic AD models may exhibit metabolic aging [42]. Therefore, HFD consumption in these

transgenic overexpressing mice might exacerbate already-present metabolic phenotypes, leading to more detrimental effects of AD-related pathology and cognitive function.

APP$^{NL-F/NL-F}$ Mouse Line - In a separate study, it was hypothesized that Aβ deposition prior to the onset of HFD consumption may be a prerequisite for exacerbated development or progression of AD-related pathologies in APP knock-in mouse models of AD [43]. Mazzei et al. (2021) used the APP$^{NL-F/NL-F}$ knock-in AD mouse model, which is similar to the APP$^{NL/NL}$ knock-in mice described above as they express APP with a humanized Aβ region under the control of the endogenous APP mouse promoter. However, the humanized Aβ sequences in APP$^{NL-F/NL-F}$ knock-in mice contain two mutations associated with AD: Swedish "NL" (KM670/671NL) and Iberian "F" (I716F). As a result, APP$^{NL-F/NL-F}$ knock-in mice start accumulating Aβ as early as at 6 months-of-age and show mild cognitive deficits by 18 months [39]. APP$^{NL-F/NL-F}$ male mice were fed a 40% HFD or a regular diet starting at 6 months-of-age for 12 consecutive weeks [43]. The authors found that HFD increases body weight, fasting glucose levels, and glucose tolerance in both APP$^{NL-F/NL-F}$ mice and their WT littermates. At 18 months-of-age, HFD-fed APP$^{NL-F/NL-F}$ mice showed increased escape latency and no preference for the target quadrant in the Morris water maze compared to HFD-fed WT mice and control diet-fed mice, indicating impaired learning and memory. Western blot analysis also revealed a decrease in the levels of the postsynaptic protein, PSD95, in HFD-fed APP$^{NL-F/NL-F}$ mice, when compared to the other three mouse groups. Additionally, 18-month-old APP$^{NL-F/NL-F}$ mice fed a HFD showed increased Aβ plaque deposition in the hippocampus as well as increased insoluble Aβ in the hippocampal extract. HFD also increased glial cell activation in APP$^{NL-F/NL-F}$ mice, but only in the stratum radiatum area of the CA1. Finally, HFD increased oxidative stress in the hippocampus of APP$^{NL-F/NL-F}$ mice.
By comparing the results reported by Salas et al. (2018) and Mazzei et al. (2021), one can conclude that HFD does not trigger AD-related pathology but instead can exacerbate existing phenotypes. However, a direct comparison between the two studies is not feasible, since diet composition and the timeline of diet administration were varied.

AD Mouse Model with APP, PS1, and MAPT Mutations:

3xTgAD Mouse Line - While many mouse models of AD focus on one aspect of AD pathophysiology – the accumulation of Aβ in the brain – there are AD mouse lines that recapitulate several key pathologies of the human condition. For example, the 3xTgAD mouse line contains mutations in APP (Swedish (K670N/M671L)), MAPT (P301L; tau), and PS1 (M146V) [44]. These mice display Aβ and tau pathology starting at 6 and 12 months-of-age, respectively. Cognitive deficits begin at 4 months-of-age [45]. Knight et al. (2014) found that consumption of 60% HFD increased the onset and severity of memory deficits in this mouse line [21]. Specifically, HFD-fed 3xTgAD mice showed a decrease in spontaneous alternations in the Y maze in later disease stages, although HFD had no effect on Y maze performance in young (3-8 month) mice. HFD-fed 3xTgAD mice at all ages showed impaired memory in the odor recognition test, a short-term rodent memory test that measures time spent exploring a novel vs familiar scent. Similarly, HFD impaired memory in the novel object recognition (NOR) test in 3xTgAD mice at all ages and the Morris water maze in 7-8-month-old mice. Interestingly, this effect is transient, as 11-16-month-old HFD-fed 3xTgAD mice no longer showed memory impairment when compared to 3xTgAD mice fed a control diet. Interestingly, despite its effect on cognitive function, HFD had no significant effect on neuropathology in this mouse line. Specifically, HFD did not affect Aβ plaque or oligomer

accumulation in the hippocampus, and the number of tau-positive cells is not significantly different between 3xTgAD mice fed a HFD or a control diet. The important observation that the authors made was that the effects of HFD on cognition can be either long-lasting or transient. Therefore, discrepancies between different studies examining the association between obesity and AD could be explained not only by the differences in AD mouse models, but also by the choice of behavioral tests and the timeline of diet consumption. Another important observation was that 3xTgAD mice appear to have metabolic changes unrelated to HFD administration. Notably, Knight et al (2014) reported that by the age of 2 months (prior to HFD administration), 3xTgAD mice have increased body weight compared to WT mice [21]. This difference disappears by 12 months, when 3xTgAD mice no longer weigh more that their WT littermates.

Rollins et al. (2018) utilized 3xTgAD mice to investigate the effect of longitudinal HFD consumption on behavior and structural changes in the brain [46]. 8-week-old 3xTgAD mice were placed on either a HFD containing 60% kcal from fat or an ingredient-matched low-fat control diet. At 25-weeks-of-age, HFD did not impair memory in 3xTgAD mice compared to control-fed 3xTgAD mice in the NOR test. In the Morris water maze, 3xTgAD mice fed a HFD show decreased learning rate across training days and spend less time near the platform in the probe trial, when compared to 3xTgAD mice fed a control diet. Therefore, HFD affects specific indexes in the Morris water maze behavioral paradigm, which might be due to the confounding factors of decreased swim speed in HFD-fed 3xTgAD mice. Magnetic resonance imaging (MRI) studies were conducted in 8-, 16-, and 24-week-old mice – at timepoints that correspond to the lack brain Aβ, the initial accumulation of intracellular Aβ, and the initiation of extracellular Aβ deposition and impaired working memory, respectively. The authors found that changes in behavior were accompanied by brain volumetric changes. 3xTgAD mice maintained on a HFD show increases in brain volume from 8- to 16-weeks-of-age. However, HFD dramatically decreases brain volume in HFD-fed 3xTgAD mice from 16 to 24 weeks-of-age. Compared to HFD-fed mice, 3xTgAD mice maintained on control diet show only localized increases in brain volume at 8-16 weeks followed by distributed decreases in brain volume at 16-24 weeks, compared to WT mice fed a control diet. In summary, in the 3xTgAD mouse model of AD, early life (4-8 weeks) consumption of a 60% HFD does not affect Aβ or tau pathology. However, it does increase gliosis and induce cognitive deficits, thus highlighting the importance of long-term monitoring of cognitive health of research rodents, since cognitive changes do not always correlate with Aβ or tau pathology.

AD Mouse Models with APOE Mutations:

APOE4 Knock-In Mouse Line - Most AD mouse models recapitulate human AD pathology as a result of mutations associated with early-onset autosomal dominant AD. However, the vast majority of AD patients do not harbor these mutations and instead present with late-onset disease. To better study late-onset AD, mouse models have been developed that carry the E4 allele of Apoe, which is the largest genetic risk factor for the late-onset, or sporadic, form of AD. In a study of Caucasian subjects, heterozygous E4 carriers were 3.2 times more likely to develop AD compared to carriers of the more ubiquitous E3 allele [47]. Moreover, homozygous E4 carriers have nearly 15 times increased risk for developing AD [47]. In APOE4 knock-in mice, endogenous mouse ApoE is replaced with human ApoE4 [48]. While these mice develop diet-induced hypercholesteremia, they do not develop AD-related phenotypes, such as Aβ deposition or cognitive decline [49]. Jones et al. (2019) administered a HFD containing 45% kcal from fat or

ingredient-matched LFD to 6-month-old APOE4 mice [50]. In this study, control animals expressed human APOE3–the ApoE allele associated with a normal risk of AD [47]. HFD increased weight gain, baseline glucose levels, glucose tolerance, and adipose tissue composition in male, but not female APOE4 mice, indicating that HFD causes a sex-dependent metabolic disturbance in APOE4 mice. HFD did not cause any robust changes in cognitive performance. Janssen et al. (2016) also evaluated the effect of a HFD enhanced with cholesterol on spatial cognition in APOE4 mice [51]. They found that HFD consumption for 3 months had no effect on learning or memory in the Morris water maze in 15-month-old APOE4 female mice, yet it decreased escape latency in WT mice, indicating improved spatial learning in WT animals. HFD also decreased CD68 immunoreactivity in the CA1 area of the hippocampus in APOE4 mice, when compared to APOE4 mice fed a standard chow, indicating HFD consumption led to decreased neuroinflammation.

Overall, chronic consumption of a HFD had no effect on cognitive function in APOE4 mice, although it increased anxiety-like behavior in one study. Given the importance of Apoe isoforms for late-onset AD, a tentative conclusion that can be made from these studies is that diet-induced obesity might not confer additional risk for AD in APOE4 carriers. However, since APOE4 mice do not develop any AD-related pathology, these results must be interpreted with caution.

APP/E4 Mouse Line - To evaluate the effect of APOE4 genotype and HFD on Aβ pathology, Nam et al (2018) crossed APOE3 or APOE4 mice to APP/PS1 mice and administered either a normal chow or a sucrose-enriched "Western" diet containing 40% kcal from fat for 3 months starting at 3.5 months-of-age [52]. HFD increased body weight and plasma cholesterol in all mouse groups. However, HFD increased Aβ deposition in the cortex and the hippocampus of APP/E4, but not APP/E3 mice, and this effect was more pronounced in females than males. RNAseq analysis of cortical samples revealed sex-specific transcriptome changes in APP/E4 in response to HFD. HFD increased the expression of genes associated with innate immune response, phagocytosis, regulation of cell migration, and positive regulation of NFkB activity and decreased the expression of genes associated with learning, long-term synaptic potentiation, and protein phosphorylation in HFD-fed female APP/E4 mice. Interestingly, in male APP/E4 mice, HFD increased the expression of genes associated with regulation of transcription and learning. To evaluate the effect of HFD on microglial activation, Nam et al. (2018) quantified the percentage of plaque perimeter contacted by microglial processes [52]. HFD significantly decreased microglia coverage in female mice only. Specifically, HFD-fed female APP/E4 mice exhibited reduced microglia around large (>200 nm^2) Aβ plaques. Overall, these results demonstrate that the effects of HFD are influenced by APOE isoform and sex to modulate Aβ deposition, microglia coverage, and cortical transcriptome changes.

In sum, a 45% "Western" HFD has detrimental effects on AD-related pathology in APP/PS1 mice carrying the APOE4 genotype. In particular, this diet increased Aβ plaque deposition and decreased microglia coverage around Aβ plaques in female APP/E4 mice, indicating an impairment in microglial function. However, since a "Western" diet combines high levels of both fat and sucrose, it is difficult to determine whether dietary fat alone has a similar effect on AD-related pathology in APP/E4 mice. Additionally, further studies are needed to elucidate how HFD affects cognitive performance in this AD mouse model.

In-Utero Studies - The evidence that chronic HFD exposure can affect AD-related pathology and cognitive function is supported by gestational HFD studies. Martin et al. (2014) exposed 3xTgAD dams to either a 60% HFD or a LFD during pregnancy and lactation and studied how in-utero exposure to HFD affected the offspring [53]. The offspring remained with their mothers for 21 days and were subsequently weaned and placed on a standard chow diet. Behavioral assessments were performed in 2-, 6-, and 12-month old female mice, since male mice already showed profound cognitive deficits at 2 months-of-age, regardless of in-utero exposure to diet. Female 3xTgAD mice from HFD-fed dams weighed significantly more at 4 weeks-of-age, when compared to female 3xTgAD from control-fed dams. However, this effect was transient, as there were no differences in the body weight between the two groups at 2 or 12 months. In the Y maze, there was no difference in the % alternation between 3xTgAD mice from HFD-fed and control-fed dams at 2- or 6 months-of-age. However, the % alternation was reduced in 3xTgAD mice from HFD-fed dams at 12 months-of-age. In the Morris water maze, mice from HFD-fed and control-fed dams were unable to learn the location of the platform in the acquisition phase of the test at 2- and 6-months-of-age. However, in the probe trial, 3xTgAD mice from HFD-fed dams spent less time in the target quadrant compared to the other quadrants, indicating impaired memory at 2 months-of-age. This difference was not observed at 6 months-of-age. Additionally, in-utero HFD exposure affected object recognition memory in 12-month-old 3xTgAD mice. In-utero HFD also increased the number of phosphorylated tau-positive neurons in the hippocampus of 12-month-old 3xTgAD mice while Aβ pathology remained unaffected. Thus, in-utero HFD exposure affected cognitive performance and increased tau pathology in a triple transgenic mouse model of AD, indicating that even short-term exposure to HFD during the critical period of development can affect AD-related phenotypes in 3xTgAD mice.

Several studies also reported the effect of in-utero HFD exposure on cognitive function in WT mice. Tozuka et al. (2010) administered either a 60% HFD or standard chow to 5-week-old male and female C57BL/6J mice for 6 weeks until mating, and female mice continued on their respective diets through pregnancy and lactation [54]. All mice were fed the standard chow starting at lactation day 16 to prevent the offspring from eating the dropped HFD before weaning. Male offspring weaned at day 22 were placed on the normal diet and were used to study the effect of in-utero HFD exposure on lipid peroxidation, generation of BDNF (brain-derived neurotrophic factor), and spatial learning and memory. At twenty-one days, HFD offspring showed greater accumulation of 4-hydroxyl hexenal, an indicator of lipid peroxidation, in neuronal cells located in the dentate gyrus of the hippocampus. These HFD offspring also had lower mRNA and protein levels of BDNF in the hippocampus when compared to mice from dams fed a normal diet. The changes in BDNF were transient, however, as there were no differences between groups at 70 days. Further studies confirmed that a decrease in hippocampal BDNF expression might be due to oxidative stress. In addition, HFD offspring showed reduced dendritic arborization of new neurons in the hippocampus and had impaired spatial learning in the acquisition phase of the Morris water maze. However, the probe trial did not reveal differences between the groups, indicating intact memory.

To further study the effect of in-utero HFD exposure on synapses in the offspring, Hatanaka et al. (2016) exposed male C57BL/6J to 60% HFD during gestation as described above and used two-photon microscopy to study the formation and elimination rates of dendritic spines and filopodia in 10-week-old mice [55]. Both formation and elimination rates of filopodia were increased by in-

utero HFD exposure, indicating that mice from HFD-fed dams had greater synaptic instability. HFD offspring also showed a persistent increased ratio of filopodia to spines. This result indicates an impairment in synaptic dynamics and morphology, since the ratio of filopodia to spines is gradually decreased in the course of synaptic development. To study how postnatal exposure to HFD affects synaptic development in mice exposed to either a HFD or a normal diet during gestation, offspring from HFD-fed and standard diet-fed dams were placed on either a HFD or a standard diet after weaning until the age of 8 weeks. The results of the two-photon imaging showed that postnatal HFD exposure induces synaptic instability similar gestational HFD exposure, and pre- and postnatal HFD exposure does not have an additive effect on the dynamics of dendritic spines and filopodia. However, a significant loss of dendritic spines was only apparent in HFD-fed mice from HFD-fed dams. Interestingly, synaptic instability was also detected in 8-week-old offspring from dams fed a normal diet during pregnancy and a HFD during lactation, indicating that even a short-term exposure to HFD during the critical period of lactation can disrupt synaptic dynamics. These effects were reversed by treatment with ascorbic acid, an antioxidant, in the drinking water. Thus, synaptic instability in mice exposed to HFD during lactation is likely due to oxidative stress. Yu et al. (2010) reported a similar detrimental effect of chronic HFD exposure on learning and memory, which was accompanied by increased serum cholesterol levels, increased brain saturated fatty acid content, and decreased brain polyunsaturated fatty acid concentration [56]. However, Yu et al. (2010) maintained the offspring of HFD-fed and control diet-fed dams on HFD or control diet from birth until behavioral analysis, which makes it difficult to elucidate the postnatal effect of HFD on cognition from its effect during gestation and lactation periods.

Overall, the results from studies focused on the effects of maternal diet in WT offspring indicate that early exposure to dietary fats may affect cognitive performance in adulthood, which is likely due to brain oxidative stress and, as a result, perturbations in synaptic development. However, future studies will need to determine whether the AD genotype can further exacerbate cognitive function and disrupt synaptic dynamics.

Protective Effects of Dietary Fat Consumption on AD Pathophysiology

AD Mouse Models with APP and PS1 Mutations:

Tg2576 AD Mouse Line - It is important to note that while many studies found a negative or neutral effect of HFD on AD-related pathology and cognitive function, there are several studies that report a protective effect of dietary fat consumption. For example, Elhaik Goldman et al. (2018) explored the effect of HFD on Tg2576 male mice, which overexpress human APP with the Swedish mutation (K670N/M671L) under the transcriptional control of the hamster prion gene promoter [24, 57]. Tg2576 mice begin accumulating extracellular Aβ as early as 6 months-of-age but show a rapid increase in Aβ plaque deposition at 10 months [58]. Spatial learning and memory deficits become apparent starting at 6 months-of-age [59]. Tg2576 mice and their WT littermates were administered either a 60% kcal HFD or a control diet from 2 to 12 months-of-age [24]. HFD induced weight gain in both WT and Tg2576 mice. However, Tg2576 mice fed a control diet gained less weight that control diet-fed WT mice. HFD also increased blood glucose levels in both WT and Tg2576 mice at 6 months-of-age but only Tg2576 mice at 11 months-of-age. As expected, HFD increased serum HDL cholesterol levels in both WT and Tg2576 mice. Interestingly, serum

cholesterol levels were lower in control-fed Tg2576 mice compared to control-fed WT mice. An open field test was used to investigate anxiety-like behavior in these mice. Compared to other groups, Tg2576 mice fed a control diet showed increased locomotion and time spent in the center of the testing arena, indicating hyperactivity and a decrease in anxiety-like behavior. HFD consumption decreased locomotion and increased anxiety-like behavior in both WT and Tg2576 mice, although the effect of HFD on anxiety-like behavior in mice could have been confounded by overall reduced activity in HFD-fed mice [24]. As expected, Tg2576 mice fed a control diet exhibited poor learning in the Morris water maze, and this learning deficit was notably rescued by HFD, since HFD-fed Tg2576 mice showed a better learning rate compared to Tg2576 mice fed a control diet. These effects were independent of Aβ pathology, since HFD did not affect the levels of cortical Aβ42. Furthermore, the effect of HFD consumption on BBB integrity was examined by MRI. Although there were no differences between groups at 4 months, there was a trend towards less extravasation in the HFD groups at 8 months. Further analysis showed a significant difference between Tg2576 mice fed control vs HFD, indicating that HFD increased the integrity of the BBB in Tg2576 mice. At 12 months-of-age, Tg2576 mice showed greater extravasation when compared to WT mice, and there was a trend for greater extravasation in mice fed a control diet when compared to HFD-fed mice. Finally, HFD significantly reduced ventricular volume in Tg2576 mice when compared to Tg2576 mice fed a control diet, indicating a protection against brain atrophy with HFD feeding. This difference was, however, no longer significant at 12 months. Taken together, these results suggest, contrary to some previous reports, that dietary fats might have a protective effect on AD-related pathology and cognitive function. A previous study evaluated the metabolic health of Tg2576 mice and concluded that this AD mouse line shows decreased weight and adiposity, low plasma leptin levels, and increased energy expenditure at 3 months-of-age, before Aβ pathology begins [60]. These metabolic alterations are accompanied by disturbances in hypothalamic leptin signaling. Metabolic disturbances and hypothalamic changes progress as Aβ burden increases. These results indicate that Tg2576 mice have a pathological metabolic phenotype that can potentially be corrected by long-term HFD consumption, protecting mice from cognitive decline as well as BBB disruption, as discussed in Elhaik Goldman et al. (2018).

In-Utero Studies - Recent evidence also shows that in-utero exposure to dietary fats might improve cognitive aging and markers of age-related pathology. Di Meco & Pratico (2019) fed B6129SF2/J WT female mice either a 42% kcal HFD or standard chow during gestation [61]. Both the lactating dams and offspring only received regular chow post-partum. The authors then investigated the effect of gestational HFD exposure on cognitive function and brain health in 18-month-old WT offspring [61]. 18-month-old WT mice that received HFD during gestation had increased body weight when compared to mice exposed to regular chow in-utero. However, there were no differences in blood glucose levels or glucose tolerance between the two groups. Although gestational HFD did not affect cognitive performance in the Y maze, HFD-exposed mice showed increased freezing behavior in the cued recall phase of the fear conditioning test, indicating improved amygdala-dependent fear memory. However, HFD did not improve hippocampus-dependent performance in the contextual phase of the fear conditioning test. In the Morris water maze, gestational HFD exposure improved learning and memory. This improvement in cognitive performance was accompanied by increased levels of postsynaptic protein PSD95 in the brains of HFD-exposed mice, indicating better synaptic integrity. WT mice exposed to HFD in-utero also showed a reduction in total tau as well as aggregation-prone tau and pathogenic tau without

changes in tau phosphorylation [61]. Finally, gestational HFD exposure decreased the activation of caspase-3, an enzyme that cleaves tau and primes it for aggregation. Thus, contrary to what has been reported by others, HFD exposure during gestation but not during lactation may exert a beneficial effect on brain health as well as on AD-related pathology and cognitive function.

In sum, some studies indicate that consumption of a HFD may be beneficial to brain health and protective against AD-related pathologies. Additional studies are still needed to determine the most advantageous HFD component(s) and consumption time-frame for providing therapeutic benefit rather than an adverse outcome.

The Effect of Genetically-Induced Obesity on AD-Related Phenotypes

The effect of obesity on AD-related pathology has also been studied in genetically-modified mice that present with a type 2 diabetes (T2D)-like phenotype. Hierro-Bujalance et al. (2019) investigated the effect of genetically-induced obesity on AD pathophysiology by crossing APP/PS1 mice with a widely used model of obesity, mice lacking a functional leptin receptor (db/db mice) [62, 63]. To compare the effect of genetically-induced obesity vs diet-induced obesity, APP/PS1 mice were fed a 60% HFD from 4 to 26 weeks-of-age [63]. Although APP/PS1xdb/db mice display increased body weight, glucose levels, and insulin levels, HFD did not significantly affect body weight or metabolic parameters in APP/PS1 mice by 26 weeks-of-age. Furthermore, while APP/PS1xdb/db mice had lower insoluble Aβ40 and Aβ42 levels in the cortex at 14 and 26 weeks-of-age compared to APP/PS1 control mice, Hierro-Bujalance et al. (2019) found that HFD consumption did not alter Aβ levels in the cortex of APP/PS1 mice [63]. Soluble Aβ levels were also increased in the cortex of APP/PS1xdb/db at 14 weeks-of-age. In the hippocampus, both APP/PS1xdb/db and HFD-fed APP/PS1 mice had higher levels of soluble Aβ40 at 26 weeks-of-age. The authors reported that genetically-induced obesity increased cell proliferation in APP/PS1 mice at 14- and 26-weeks-of-age. This effect was not, however, observed in HFD-fed APP/PS1 mice. Both APP/PS1xdb/db and HFD-fed APP/PS1 mice also exhibited an increase in Brdu+ cells in the cortex, but not throughout the hippocampus, at 26 weeks-of-age, indicating increased site-specific neurogenesis. Interestingly, at 26 weeks-of-age APP/PS1xdb/db mice showed an increase in Brdu+ cells in the dentate gyrus of the hippocampus, which is recognized as a major neurogenic niche.

Hierro-Bujalance et al. (2019) did not address how the db/db genotype affects cognitive dysfunction in APP/PS1 mice, but a previous study performed a similar experiment by crossing a transgenic AD mouse model with another mouse model of genetic-induced obesity – ob/ob mice [64]. Unlike db/db mice, which lack a functional leptin receptor, ob/ob mice are characterized by a mutation in the gene encoding leptin, rendering ob/ob mice leptin-deficient [65-67]. Takeda et al. (2010) crossed APP23 transgenic mice with ob/ob mice [64]. APP23;ob/ob mice showed early-onset obesity and a severe diabetic phenotype, including hyperglycemia, hyperinsulinemia, hyperlipidemia, and glucose intolerance. Interestingly, the diabetic phenotype was more pronounced in APP23;ob/ob mice when compared to ob/ob mice, and ob/ob mice were slightly leaner. APP23;ob/ob mice showed a deficit in learning and memory in the Morris water maze as early as at 8 weeks-of-age, before APP23 mice exhibited cognitive impairment. Interestingly, there was no Aβ accumulation in these mice at this time point, suggesting that cognitive dysfunction induced by the ob/ob genotype cannot be explained by increased Aβ accumulation. However, 12-month-old APP23;ob/ob mice exhibited increased CAA, while APP23 mice had only faint

microvascular deposits at the same age. CAA was accompanied by cerebrovascular inflammation, as the ob/ob genotype induced the expression of receptor for advanced glycation end products (RAGE) which, in turn, induced the expression of potent inflammatory markers Il-6 and TNFα in APP23 mice. Overall, the results from both ob/ob and db/db mouse experiments suggest disturbances in leptin signaling have a detrimental effect on AD-related pathophysiology and cognitive function in APP/PS1 and APP23 mice.

Another study described a protective effect of genetically-induced obesity and T2D on AD-related pathology [20]. Wakabayashi et al. (2019) compared the effect of HFD-induced insulin resistance vs genetically-induced insulin resistance on the development of AD-like pathology in transgenic A7 mice (A7-Tg) [20]. A7-Tg mice overexpress mutant human APP containing Swedish (K670N/M671L) and Austrian (T714I) mutations under the control of neuronal-specific Thy-1 promoter [68]. As a result of transgenic overexpression, these mice develop robust Aβ pathology in the brain by approximately 9-12 months of age. Wakabayashi et al. (2019) administered a 32% kcal HFD to A7-Tg mice and their WT littermates starting at 3 months-of-age [20]. HFD consumption had no effect on either soluble or insoluble Aβ in brain homogenates of 5-month-old mice, but increased both soluble and insoluble Aβ levels in brain homogenates of 9-month-old A7-Tg mice. Finally, at 15 months-of-age, after 12 months of HFD feeding, A7-Tg mice showed increased Aβ deposition in the brain compared to control A7-Tg mice. However, these effects were not observed in A7-Tg mice lacking insulin receptor substrate-2 (IRS-2) [20]. IRS-2-deficient mice develop T2D-like insulin resistance and pancreatic β cell dysfunction. $IRS2^{-/-}$;A7-Tg mice have increased body weight and elevated fasting blood glucose levels, a phenotype that resembles HFD-induced abnormalities. However, IRS-2 deletion appears to be protective against AD-related pathology, since $IRS2^{-/-}$;A7-Tg mice display lower soluble and insoluble Aβ40 and Aβ42 at 9 months-of-age, when compared to $IRS2^{+/+}$;A7-Tg mice. By 15 months, $IRS2^{-/-}$;A7-Tg mice also show decreased Aβ deposition in the brain compared to $IRS2^{+/+}$;A7-Tg mice. However, when $IRS2^{-/-}$;A7-Tg mice are fed the above described HFD, they display increased soluble Aβ40 and Aβ42 at 10 months-of-age as well as increased cortical Aβ deposition at 15 months-of-age, a sharp reversal in the protective effect induced by IRS-2 deletion. Overall, the results of this study demonstrate that the association between diet consumption, insulin signaling, and AD pathophysiology is complex, and the effects of diet-induced obesity on AD-like pathology in mouse models cannot be explained by impaired insulin signaling alone. Additionally, genetically-induced obesity can exert either a detrimental or a protective effect on AD-related pathophysiology, depending on the genetic alteration causing the weight gain: $IRS2^{-/-}$ mice lack IRS-2, a protein that plays a key role in eliciting many of insulin's actions, while db/db and ob/ob mice have deficient leptin signaling. These results highlight the complex association between obesity and AD, since both conditions are mediated by several key factors.

Limitations

As evidenced from the studies described above, some reports focused on either male or female mice when investigating the effect of HFD on AD-related pathology and cognitive function [21, 69], while some studies pooled male and female mice in order to increase sample size and not determine if sex is an independent variable [50]. This difference can be considered a signification limitation of the studies, since emerging evidence suggests that the effects of HFD on peripheral metabolism as well as AD-related pathology and cognitive function are, at least in part, sex-dependent.

While Knight et al. (2014) investigated the effect of HFD on male 3xTgAD mice and reported that HFD-fed 3xTgAD mice exhibit increased onset and severity of cognitive deficits without changes in AD-related pathology [21], Sah et al. (2017) focused on HFD-induced changes in AD-related pathophysiology in female mice, which exhibit more noticeable brain Aβ deposits when compared to age-matched males [69]. Specifically, Sah et al. (2017) administered a 60% kcal HFD for 16 weeks starting at 4 weeks-of-age [69]. HFD-fed female 3xTgAD mice showed impaired learning and memory in the Morris water maze. Interestingly, the levels of cortical Aβ oligomers and phosphorylated tau were not altered by either the diet or the genotype in these young mice, which is similar to the results Knight et al. (2014) reported in age-matched as well as older 3xTgAD male mice [21]. However, Western blot analysis of cortical tissue showed evidence of oxidative stress and neuronal apoptosis, which might underlie the cognitive deficits induced by HFD in female 3xTgAD mice in the absence of enhanced Aβ accumulation.

Robison et al. (2020) administered a 60% kcal HFD or a low-fat control diet to male and female 3xTgAD mice or WT mice starting at 3-months-of-age [70]. All mice remained on the diet for 4 months. On control diet, 3xTgAD male mice weighed less than WT males, but the opposite was observed in that female 3xTgAD mice weighed more that WT female mice. Similarly, 3xTgAD male mice on control diet had less subcutaneous and visceral fat than both WT males and 3xTgAD female mice. Both male and female WT and 3xTgAD mice fed the HFD gained more weight and had more subcutaneous and visceral fat than mice fed a control diet. However, HFD-fed 3xTgAD female mice gained a higher percentage of weight and exhibited more subcutaneous and visceral fat than HFD-fed WT females or HFD-fed 3xTgAD male mice. Interestingly, weight gain in HFD-fed 3xTgAD female mice was accompanied by reduced activity in the open field test as well as reduced thigmotaxis, indicating a decrease in anxiety-like behavior. As expected, HFD impaired glucose tolerance in all groups. However, the greatest degree of impairment was observed in 3xTgAD female mice. 3xTgAD female mice fed a control diet had impaired glucose tolerance when compared to female WT mice fed a control diet, which is in line with the finding that, on the control diet, 3xTgAD female mice weighed more than WT females. Similarly, female mice had greater hepatic steatosis and ballooning, indicating more advanced non-alcoholic fatty liver disease (NAFLD), and this difference was most pronounced in animals fed a control diet. HFD increased steatosis and inflammation in both males and females, although ballooning was evident in male mice only. Interestingly, male 3xTgAD mice were particularly susceptible to HFD-induced NAFLD. 3xTgAD mice overall had greater HFD-induced fibrosis compared to WT mice, although females had greater fibrosis than males. As expected, HFD induced hyperleptinemia, and female 3xTgAD mice had higher leptin levels that WT mice. Interestingly, 3xTgAD male mice fed a control diet exhibited extremely low levels of leptin in plasma. Conversely, HFD reduced ghrelin levels in mice [70]. Systemic inflammation, including increased expression of IL-10 and IL-12, was also increased in 3xTgAD mice, especially in males. Similarly, 3xTgAD mice had higher expression of Iba1 in the hypothalamus, although this effect was primarily driven by 3xTgAD male mice that had higher Iba1 expression than both WT males and 3xTgAD females. While 3xTgAD male mice fed a control diet had higher hypothalamic GFAP protein expression than their female counterparts, female 3xTgAD mice fed a HFD had higher GFAP levels when compared to all other groups. At the age selected in this study (7 months), 3xTgAD mice showed no evidence of Aβ deposition in the hypothalamus. Thus, observed changes in microgliosis and astrogliosis were independent of Aβ pathology.

Martins et al. (2017) used the same transgenic AD line to determine the effect of HFD on AD-related phenotypes in male and female mice, focusing largely on behavioral and mitochondrial abnormalities [71]. They administered a HFD containing 60% kcal from fat to male and female 3xTgAD and WT mice starting at 8 weeks-of-age. Mice remained on their respective diets for either 2, 6, or 12 months. HFD induced a significant weight gain in male and female mice at all ages. However, male 3xTgAD mice gained less weight than their WT counterparts following HFD consumption, consistent with the results reported by Robison et al. (2020) [70]. Interestingly, HFD did not affect blood glucose levels in male or female 3xTgAD or WT mice in any age group. However, at 4 months-of-age, plasma insulin levels were increased in HFD-fed male WT mice, but not female WT mice or either male or female 3xTgAD mice. By 14 months-of-age, HFD increased blood insulin levels in both male and female WT mice, while this effect was not observed in 3xTgAD male or female mice. HFD accelerated memory decline in the Y maze in both male and female 3xTgAD mice at 8-months-of-age. Interestingly, by 14 months-of-age HFD also impaired memory in WT male and female mice, as HFD-fed WT mice showed fewer spontaneous alternations in the Y maze. In 14-month-old 3xTgAD, there was no effect of diet on the performance in the Y maze. However, control diet-fed female 3xTgAD mice performed worse than their male counterparts at 14 months-of-age. Deteriorated cognitive performance in HFD-fed WT mice was accompanied by a reduction in hippocampal synapses. Finally, HFD consumption led to mitochondrial elongation in the capillary endothelium in WT mice, mirroring changes observed in the endothelium of 3xTgAD mice fed a control diet. These changes in morphology were not accompanied by changes in mitochondrial number. However, the number of mitochondria was reduced in the neuropil of the hippocampus in response to a HFD in WT mice and in 3xTgAD mice fed a control diet. Martins et al. (2017) did not investigate the effect of HFD on Aβ or tau pathology, as they had previously observed that HFD-induced memory deficits were independent of Aβ deposition and tau phosphorylation [21].

Taken together, the results of the studies described above highlight that HFD might affect male and female mice differently. Using mice of only one sex or pooling together data from male and female mice for the sake of increasing statistical power can lead to results that are inconclusive or difficult to interpret.

Another potential limitation of the studies summarized in this here is comparing results obtained from HFD-fed mice to results obtained from mice fed standard grain-based diets, usually referred to as “normal chow”, “regular chow”, or “standard diet”, as opposed to ingredient-matched control diet. Pellizzon & Ricci (2018) reported that standard chow diets often contain ground corn, ground wheat, ground oats, fish meal, and other unrefined ingredients [72]. In addition to micronutrients provided inherently from these ingredients, grain-based diets also contain premixes of vitamins and minerals. However, the formula and the levels of nutrients of grain-based diets may be kept proprietary by vendors and change over time. In addition, grain-based diets often contain non-nutrients, including pesticides, heavy metals, genetically-modified grains, polychlorinated biphenyls, polychlorinated dibenzo-p-dioxins, and dibenzofurans [73]. In contrast, purified ingredient control diets use refined ingredients (casein, corn starch, sucrose, cellulose, soybean oil, etc.) and have minimal batch-to-batch variability and few of any non-nutrient chemicals. Moreover, they match HFDs in terms of the sources of macronutrients as well as added vitamins and minerals. However, in several papers discussed here [29, 33, 34, 38, 43], phenotypic differences attributed to HFD consumption may be due to any number of dietary differences, not

only the fat content of the diet. This is a pervasive issue that stems from the low cost of standard grain-based mouse chow. In fact, Pellizzon & Ricci (2018) report that in 41% of studies they surveyed in 2016, data from mice fed a HFD were improperly compared to those fed a non-purified grain-based diets, or normal chow [72].

Another significant limitation is the comparison between transgenic mice and WT mice of the same strain that are not genetically identical or that have not been subject to the same environmental conditions. To determine the effect of HFD on AD-related pathology in mouse models of AD, Lin et al. (2016) used 5XFAD mice on the congenic C57BL/6J background. However, instead of using WT littermates, Lin et al. (2016) compared 5XFAD to newly purchased WT C57BL/6J mice. C57BL/6 strains differ significantly, and the differences between various B6 substrains are often larger than the differences between C57Bl/6 mice and other inbred strains such as B10 [74]. In addition, using strains from other colonies means that the mice also differ epigenetically and are subject to environmental factors that cannot be effectively controlled. The best solution to this problem is using WT littermates, ensuring equal genetic and environmental influences.

Another drawback that may affect how data are interpreted in these HFD studies is the influence of the background strain on mouse phenotypes. In at least two of the studies summarized here, the authors utilized the 5XFAD mouse model on the hybrid C57BL/6 x SJL background to study how HFD affects AD-related pathology and cognitive function [25, 33]. Because this model is maintained by backcrossing transgenic animals to a B6SJLF1 hybrid at every generation, C57BL/6J and SJL/J content is segregating in the progeny of these animals. According to Jackson Laboratory, mice produced from this cross could be genotypically heterozygous, homozygous, or WT for various mutations, including the $Trem2^{S148E}$ allele. To minimize concerns related to allele segregation and the high variability of the original hybrid background, the 5XFAD line was also made on the congenic C57BL/6J background. However, both lines are still in use. Thus, when interpreting the results of HFD studies in 5XFAD mice, one must be conscious of the influence that genetic background might have on the observed phenotypes.

Finally, a major limitation of these studies is the variability between AD mouse lines used. Various transgenic mouse lines express mutated human *APP* and/or *PS1* and/or *MAPT* under the control of various promoters, introducing a significant degree of variability into the pathophysiology, expression levels and expression patterns of pathogenic proteins, and the progression of cognitive decline. In addition, some models are poorly characterized in terms of their sensitivity to behavioral testing, the amount of AD-related pathology, and the extent of synaptic damage, making absolute comparisons between models difficult. The development of knock-in AD mouse lines solved some of the issues associated with transgenic AD mouse lines, as they express mutated human *APP* at physiological levels and under the control of the endogenous mouse promoter. However, it is important to point out that that AD-related pathology and cognitive decline in knock-in AD lines develops only after the knock-in of a combination of multiple mutations, which only occur in a very small subset of patients with the familiar form of the disease and which typically do no co-occur in single patient. Thus, with AD being almost a uniquely human disease, it is important to further validate the most relevant AD mouse models in order to address the impact of HFD on AD pathophysiology in a way that would be meaningful for clinical practice.

In conclusion, calorically dense diets, specifically those high in fat, provide an opportunity to model diet-induced obesity and investigate the effect of dietary fats on AD-related pathology and cognitive dysfunction in AD mouse lines. However, due to the 1) diversity of AD mouse models, 2) wide range of commercially available high fat diets, 3) feeding protocols that vary the timelines of HFD consumption, and 4) sex differences in both diet-induced obesity and AD pathophysiology, published studies have failed to yield conclusive results. While many studies report HFD-induced amelioration of AD-related pathology and increased onset and severity of cognitive decline, others report no association between HFD and AD. Moreover, recent studies show that HFD can exert a beneficial effect on AD-related pathologies. Additional studies are necessary to uncover the mechanisms that underlie both the detrimental and beneficial effects of HFD consumption and help translate the findings into actionable clinical strategies.

The goal of this study was to characterize the effect of HFD on AD-related pathology and cognitive function in mice over various timelines. Tg6799 (AD) mice and their wild type (WT) littermates were fed either HFD or control (CON) diet. We determined the diet's effect on Aβ plaque load in the retrosplenial cortex (RSC) and hippocampus of AD mice as well as cognitive function. We also studied the effect of HFD on the extravasation of fibrinogen into the brain, a pro-inflammatory plasma protein that is involved in AD pathogenesis [75]. We show that when HFD was fed to AD mice early in life, there was less parenchymal Aβ deposition, fibrinogen extravasation into the brain, and cognitive dysfunction compared to AD CON mice. However, this neuroprotection was not observed in AD mice that started consuming a HFD later in life, after AD pathology was already established. These results suggest that at early stages of AD, dietary fats may be protective to the brain and slow AD onset and progression.

Chapter 2: Materials and Methods

Animals

Male Tg6799 mice [30] (referred to as AD mice) and their wildtype (WT) littermates were housed with food and water ad libitum, under controlled temperature (20-22°C), humidity (40-60%), and illumination (12/12h dark cycle). Experimental diets were administered starting at either 1, 3, or 6 months-of-age. All animal experiments were conducted in accordance with the guidelines of the US NIH Guide for the Care and Use of Laboratory Animals and with approval from the Animal Care and Use Committee of The Rockefeller University.

Diet-induced obesity model

AD mice and their WT littermates were divided into four groups: AD CON, AD HFD, WT CON, and WT HFD. HFD contained 60% kcal from fat, 20% kcal from carbohydrates, and 20% kcal from protein (D12492, Research Diets Inc.), and CON contained 10% kcal from fat, 70% kcal from carbohydrates, and 20% kcal from protein (D12450J, Research Diets Inc.). HFD contained 0.03% cholesterol, while CON contained 0.005% cholesterol. Mice were weighed weekly, and body composition was assessed by EchoMRI-100H (EchoMRI, LLC). Mice remained on their respective diets during behavioral testing. At the conclusion of diet administration, mice were sacrificed and tissue was collected for analysis.

Fasting blood glucose levels and intraperitoneal glucose tolerance test

Fasting blood glucose levels and glucose tolerance were measured in 6-, 8-, or 11-month-old mice. In short, mice were fasted overnight in clean cages with free access to drinking water. The following morning, two drops of blood were collected from the tail vein, and fasting glucose levels were measured using a handheld glucometer (Contour Next EZ Glucose Meter, Bayer). Glucose (2 g/kg body weight in 0.1 ml sterile water) was then injected intraperitoneally, and blood glucose levels were measured at 5, 15, 30, 60, 90, and 120 min post injection. At the end of the experiment, mice were returned to their cages with their experimental diet provided ad libitum.

Blood ketone levels

Fasted blood ketone levels were measured in 6-, 8-, or 11-month-old mice after fasted blood glucose levels were measured. Mice were fasted overnight with access to drinking water. The following morning, two drops of blood were collected from the tail vein, and blood ketone levels were measured using a handheld ketone meter (Precision Xtra Blood Glucose and Ketone Monitoring System, Abbott). We repeated blood ketone level measurements after mice were re-fed, at least 24 hours after the fasting period ended.

Blood collection and plasma preparation

Blood was collected by cardiac puncture using a sterile 25G x 5/8 needle and EDTA-coated 1 mL syringe. Blood was transferred into EDTA-coated tubes (SAI Infusion Technologies) and centrifuged at 1500 x g for 15 min at room temperature (RT). The supernatant was transferred into polypropylene tubes (Fisher Scientific) and centrifuged again as described above. Plasma was then aliquoted into clean polypropylene tubes and stored at -80°C until use.

Cholesterol analysis
Plasma samples were submitted to the Laboratory of Comparative Biology at Memorial Sloan Kettering Cancer Center (MSKCC) where total cholesterol, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) were measured spectrophotometrically using a Beckman Coulter AU680 analyzer.

Behavioral analysis
Novel object recognition (NOR) was performed during the dark phase of the light-dark cycle to allow these nocturnal animals to carry out cognitive tasks when they are most active. NOR was carried out for 4 days with a 5-min trial on each day. The first two days consisted of habituation trials where mice were allowed to freely explore the testing arena - a large opaque rectangular box (54.9cm x 39.3cm x 32cm) with a sawdust-covered bottom. On the training day, mice were allowed to explore two identical objects (Intact Tissue-Tek O.C.T. Compound Bottles, Cat No. 25608-930) inside the testing arena. On the testing day, one of the objects was replaced with a novel one (stacked DUPLO® blocks), and the mice were allowed to interact with both objects. In order to prevent animals from using any olfactory cues, the objects and the box were cleaned with 20% ethanol after each trial. Object exploration was defined as the length of time the animal spent sniffing or touching the surface of the object with its nose and/or forepaws within a distance of 1 cm from the object. The time spent exploring both objects during training and testing was used to calculate the novel-to-familiar object exploration ratio. A ratio >1 indicates a preference for the novel object. A clear preference for one of the objects indicates that the animal could remember which of the two objects had previously been explored. We also examined locomotor ability of all animals by determining total distance moved and velocity to control for the potential confounding effect of obesity on object exploration. The behavioral performance was recorded and tracked for automated analysis of locomotion using EthoVision XT software (Noldus). Object exploration behavior was scored manually by two investigators who were unaware of mouse genotypes using EthoVision XT software.

Immunofluorescence
Mice were deeply anesthetized by intraperitoneal (IP) injection of 2.5% tribromoethanol and perfused with 0.9% ice cold saline containing heparin. For fixed brain tissue, brains were removed and the left hemisphere was fixed in 2% paraformaldehyde (PFA) in 0.1 M phosphate buffer (PB) for 24 hours at 4°C with gentle agitation. Brains were then dehydrated in 30% sucrose in PBS for 48 h at 4°C, rapidly frozen on dry ice, and sectioned at a thickness of 30 μm using a microtome. Sections were collected into PBS, transferred into cryoprotectant (30% glycerol, 30% ethylene glycol, 40% 0.1 M PB), and stored at -80°C. For analysis, sections were quickly rinsed in PBS, mounted onto slides using a gelatin-based mounting medium (0.35% gelatin, 20% ethanol, and 0.04% sodium azide in PBS), and dried overnight. The next day, sections were washed in PBS. For frozen brain tissue, brains were removed, and the left hemisphere was rapidly frozen on dry ice and stored at -80°C. Brains were sliced into 30 μm sections using a cryostat, thaw-mounted onto slides, and stored at -80 °C until use. All sections were blocked in 5% normal donkey serum with 0.3% Triton X-100 for 1 hour at RT. Prior to staining, sections were allowed to adjust to RT and fixed in 50% ethanol/50% methanol solution for 10 min at -20°C. Primary antibodies were diluted in 3% normal donkey serum with 0.3% Triton X-100 and 0.02% sodium azide, and sections were incubated with primary antibody overnight at 4°C. The following antibodies and dilutions

were used: mouse anti-6E10 Alexa Fluor 488 (1:1000, BioLegend), rat anti-CD11b (1:10, DSHB), rabbit anti-fibrinogen (1:500, Dako), and goat anti-collagen IV (1:500, Millipore). The following day, sections were incubated with an appropriate fluorescent secondary antibody (Alexa Fluor, donkey anti-host species, Life Technologies) for 1 hour at RT. Coverslips were added using Vectashield Mounting Medium (Vector). Sections were imaged at 20x magnification using Eclipse Ti2-E inverted epifluorescence microscope (Nikon) or at 63x magnification using LSM 880 Airyscan NLO inverted laser scanning confocal microscope (Zeiss). Images had consistent exposure time and gain for each stain between all sections from all animals, and only identical brain regions were compared between groups. To compare Aβ plaque load across several cohorts of mice, brain slices obtained from 6-, 8-, and 11-month-old mice were stained and imaged simultaneously. Exposure time, gain, and threshold settings were kept consistent for all cohorts. Images were thresholded manually using NIS-Elements software (Nikon Instruments, Inc.) to subtract background from apparent positive signal; threshold settings were kept consistent for all experimental images. The intensity of the signal was quantified as percent area for each section. Positive areas from each section of each animal were then averaged and compared between groups using GraphPad Prism software.

Immunohistochemistry

Mouse epididymal white adipose tissue (WAT) was used for fibrinogen immunohistochemistry. After mouse perfusion, WAT was removed and placed in 10% formalin for 24 hours at RT and then transferred to 70% ethanol for 24 hours at RT. Fixed WAT was embedded in paraffin, sectioned at a thickness of 5 μm using a microtome, and mounted onto glass slides. Glass slides were placed in a 58°C oven for 1.5 hours to melt the paraffin. Tissue sections were then re-hydrated, incubated in Proteinase K solution at 37°C for 20 min to unmask epitopes, washed in TBS, and blocked in 10% normal goat serum 5% BSA solution. Tissues were incubated in rabbit anti-fibrinogen antibody (1:200, Dako) at 4°C overnight. The next day, tissue sections were washed and incubated in biotinylated anti-rabbit antibody (1:200, Vector Laboratories) for 1 hour at RT. The staining was developed using the Vectastain ABC kit (Vector Laboratories) and the DAB Peroxidase Substrate Kit (Vector Laboratories) according to manufacturer's instructions. To visualize cell morphology, tissue sections were stained using hematoxylin. Coverslips were added with Vectamount AQ Aqueous Mounting Medium (Vector Laboratories). Images were collected on Revolve upright epifluorescence microscope (Echo), and fibrinogen staining was quantified using Fiji (NIH).

Western blot

Mice were deeply anesthetized by IP injection of 2.5% tribromoethanol and perfused with 0.9% ice cold saline containing heparin. Mouse cortical tissue was dissected and stored at -80 ^{0}C before protein extraction. Protein extraction was performed as reported previously **[76]** with modifications. In brief, cortical samples were homogenized in QIAzol lysis reagent (Qiagen) using a hand-held tissue homogenizer (Polytron PT 1200E; VWR) and incubated at room temperature for 3 min with added chloroform. Samples were centrifuged at 12000 x g for 15 min at 4 °C. The aqueous phase was removed for RNA isolation, and DNA was precipitated with ethanol. Isopropanol was added to the remaining supernatant, and the samples were centrifuged at 12000 x g for 15 min at 4 °C. Protein pellets were washed twice with 95% ethanol, homogenized in RIPA lysis buffer (Millipore, Cat No.20-188) containing a protease inhibitor cocktail (Sigma-Aldrich),

and sonicated. Total protein concentrations were measured using Pierce BCA Protein Assay Kit (Thermo Scientific). Protein samples (10 μg) were boiled at 95 °C with 4x Laemmli sample buffer for 5 min. Samples were separated on 4-20% Criterion TGX Stain-Free Protein Gel (Bio-Rad) and transferred to 0.2 μm PVDF (polyvinylidene difluoride) membrane using Trans-Blot® Turbo™ Transfer System (Bio-Rad). After blocking with 5% BSA in Tris-buffered saline with 0.1% Tween 20 for 1 hr at RT, membranes were incubated overnight at 4°C with purified anti-β-amyloid antibody (6E10, BioLegend, 1:1000) and HRP-conjugated anti-GAPDH monoclonal antibody (Proteintech, 1:10,000). Blots were further incubated with secondary antibody (HRP-conjugated sheep anti-mouse IgG; GE Healthcare, 1:10,000) for 2 h. Bands were detected via Clarity Western ECL substrate (Bio-Rad) and scanned using ChemiDoc Imaging System (Bio-Rad). After target bands were detected, band intensity was analyzed using NIH Image J software. Proteins were normalized to GAPDH, used as a loading control.

Plasma kallikrein activity assay

Plasma kallikrein activity was measured as described in [77] with some modifications. Briefly, plasma samples were diluted 1:20 in HEPES-buffered saline (20 mM HEPES, pH 7.4, 140 mM NaCl) and mixed with a chromogenic substrate, S-2302 (0.67 mM final concentration). Absorbance at 405 nm was recorded for 30 min at 37°C using a spectrophotometer (Molecular Devices). Samples were run in duplicate.

RNAseq

To perform an unbiased screen of transcriptome changes in the brain induced by diet in WT and AD mice, we purified RNA from the cortex using RNeasy mini kit (Qiagen). We then assessed the quality of purified RNA using the Bioanalyzer (Agilent Technologies) and excluded all samples with RNA integrity number < 8. We generated sequencing libraries using the TruSeq Stranded mRNA Library Prep set (Illumina) and performed RNAseq on Illumina NextSeq 500 (Illumina) at Rockefeller's Genomics Resource Center.

Data analysis

Statistical analysis was conducted using GraphPad Prism software. Data are presented as mean ± SEM. Immunofluorescence results were analyzed using unpaired t-test, while all other experimental results were analyzed using two-way ANOVA. Gene expression was analyzed using two-way ANOVA with diet as a two-level factor (HFD, CON) and genotype as a two-level factor (AD, WT), and we tested the interaction between diet and genotype. DESeq2 was used for normalization and differential expression analysis of RNA-Seq data. Post hoc analyses were conducted using Sidak's multiple comparison test. Significance threshold was set to $p \leq 0.05$.

Chapter 3: The Effect of Early Dietary Fat Supplementation on AD-related Pathology and Cognitive Function

HFD induced weight gain and affected body composition in 6-month-old mice.

To investigate the effect of increased dietary fat intake on AD-related pathology in mice, we placed 4-5-week-old mice on a diet of 60% kcal from fat (HFD) or an isocaloric control diet with 10% kcal from fat (CON) for 20 weeks until the mice reached 6 months-of-age (Fig. 1A; Cohort I). HFD induced significant weight gain in both WT and AD mice (Fig. 1B, C). HFD also affected body composition and induced a significant increase in percent body fat mass and a decrease in percent lean mass in HFD-fed WT and AD mice (Fig. 1D, E). HFD impaired the ability to clear circulating glucose after a glucose tolerance test (GTT) in WT mice (Fig. 2A, B). HFD-fed AD mice showed an increase in blood glucose after the GTT test when compared to CON-fed AD mice (Fig. 2A, B). Additionally, HFD induced hyperglycemia after a period of fasting in both WT and AD mice (Fig. 2C). Fasting also induced an increase in blood ketones in HFD-fed WT but not AD mice (Fig. 2D). However, after refeeding, both WT HFD and AD HFD mice showed an increase in blood ketones when compared to CON-fed mice (Fig. 2E). Plasma levels of cholesterol were significantly increased in HFD-fed WT and AD mice (Fig. 2F). Although HFD induced a small increase in plasma high-density lipoprotein (HDL) levels, the difference between HFD-fed and CON-fed mice was not significant (Fig. 2G). HFD did not affect plasma HDL levels in AD mice (Fig. 2G). Neither AD genotype nor HFD affected plasma levels of low-density lipoprotein (Fig. 2H).

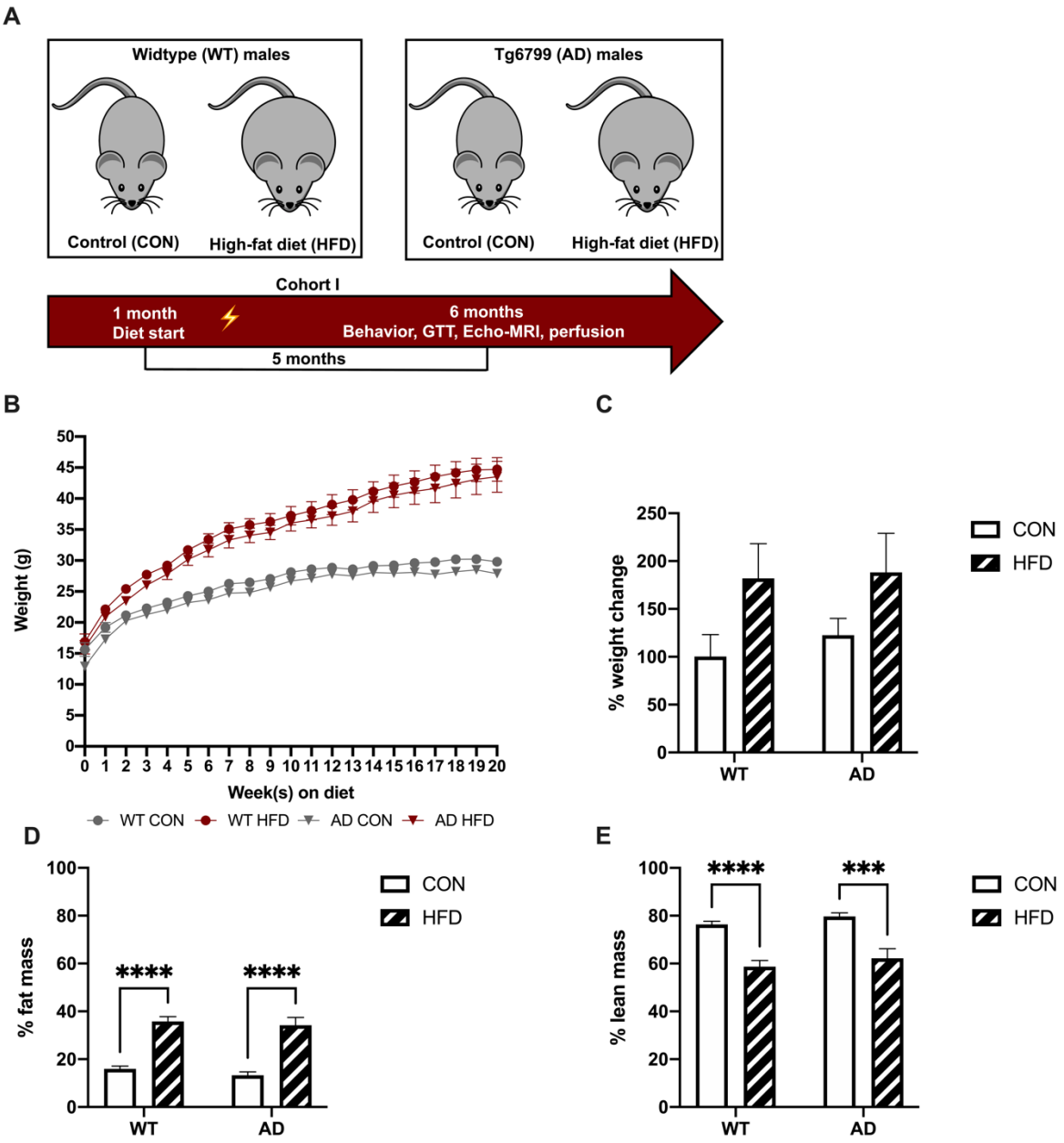
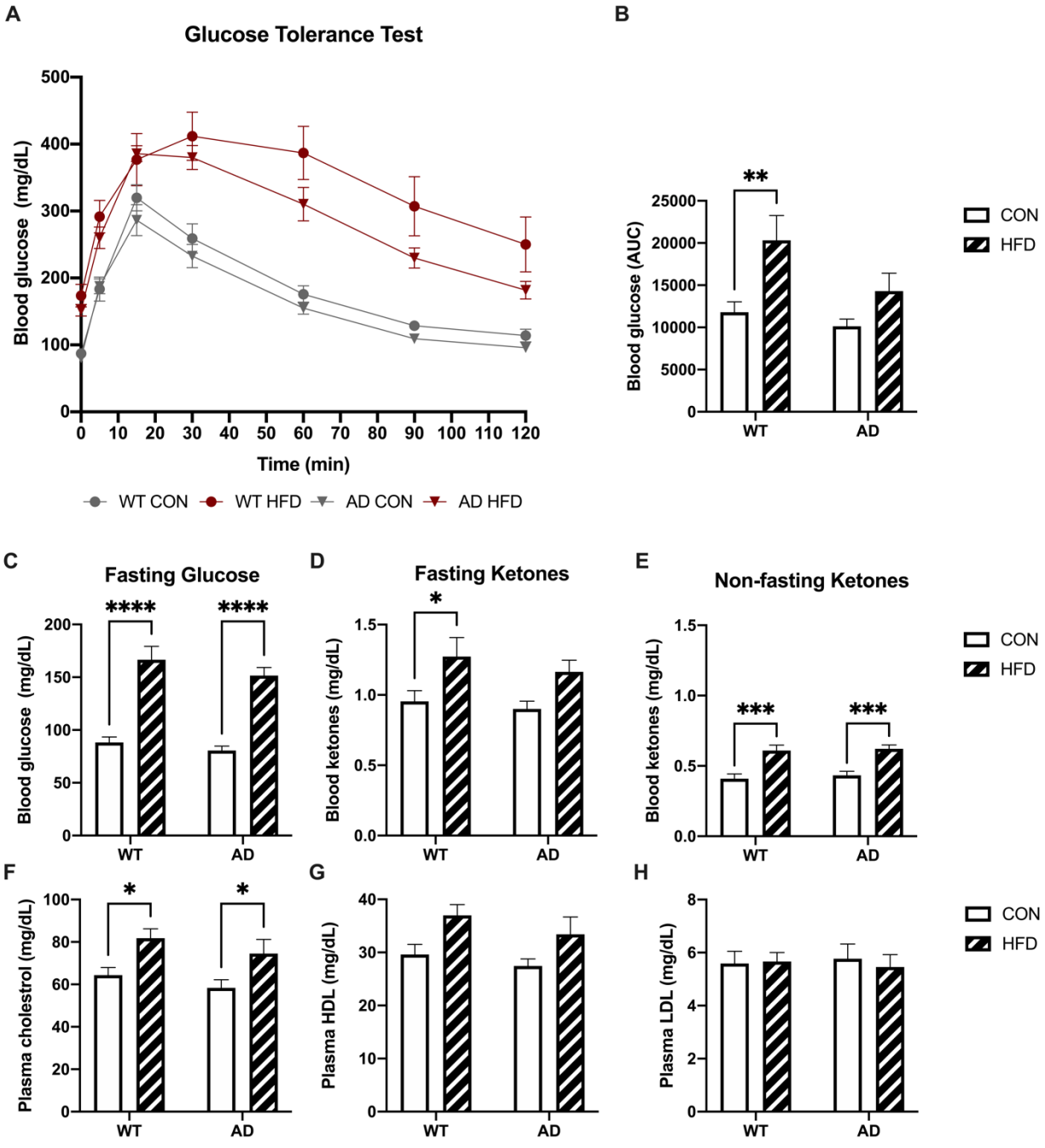


Figure 1. HFD caused significant weight gain and affected body composition similarly in WT and AD mice.

A Schematic shows feeding timeline in WT and AD mice in Cohort I. The lightning icon indicates the onset of AD-related pathology. **B** Male mice (4-5 weeks old) fed a HFD vs CON had greater weight gain. **C** HFD induced a significant increase in % weight change ($p<0.05$), but post hoc analysis did not reveal statistically significant differences between groups. **D** HFD increased % fat mass in WT and AD mice. **E** HFD decreased % lean mass in WT and AD mice. n=9-11 mice per group. Results are from one representative experiment. Statistical analysis performed by two-way ANOVA. ****$p<0.0001$.

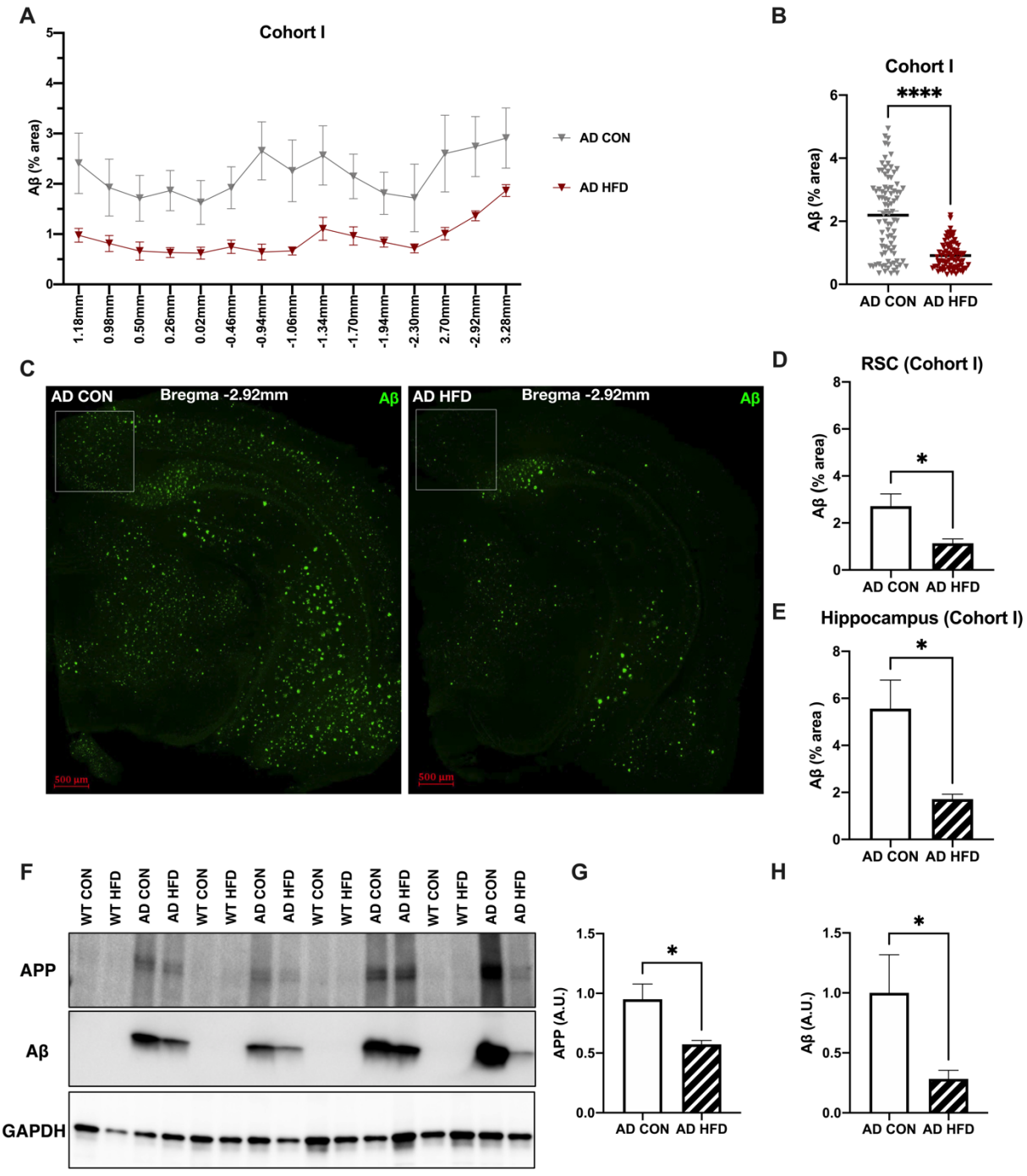
Figure 2. HFD induced hyperglycemia and hyperlipidemia, affected glucose metabolism, and caused ketogenesis in 6-month-old mice.
A HFD impaired glucose clearance in WT and AD mice in a glucose tolerance test (GTT). **B** HFD-fed WT mice showed higher blood glucose levels after GTT, compared to CON-fed mice, as indicated by area under the curve (AUC). **C** HFD increased blood glucose levels in fasted WT and AD mice. **D** HFD increased blood ketone levels in fasted WT, but not AD mice. **E** HFD increased blood ketones in non-fasted WT and AD mice. **F** HFD increased the level of plasma cholesterol in WT and AD mice. **G, H** HFD induced a small, non-significant increase in plasma HDL levels but not plasma LDL levels in WT and AD mice. For **A-C, F-H**: n=8-11 mice/group. Results are from one representative experiment. For **D, E**: n=12-14 mice/group. Results are from two independent experiments. Statistical analysis performed by two-way ANOVA. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.


A
Glucose Tolerance Test
Blood glucose (mg/dL)
Time (min)
WT CON
WT HFD
AD CON
AD HFD
B
Blood glucose (AUC)
**
CON
HFD
WT
AD
C
Fasting Glucose
Blood glucose (mg/dL)
****
****
D
Fasting Ketones
Blood ketones (mg/dL)
*
E
Non-fasting Ketones
Blood ketones (mg/dL)
***
***
F
Plasma cholestrol (mg/dL)
*
*
G
Plasma HDL (mg/dL)
H
Plasma LDL (mg/dL)

HFD consumption starting at 1 month-of-age reduced Aβ pathology in 6-month-old AD mice. HFD significantly reduced percent area covered by Aβ plaques throughout the brains of 6-month-old AD mice when compared to AD CON mice (Fig. 3A, B). Aβ plaque coverage was also specifically quantified in the RSC (boxed area, Fig. 3C) and hippocampus at Bregma -2.92 mm of all AD CON and AD HFD mice (Fig. 3C, D). HFD significantly reduced percent area covered by Aβ plaques in the RSC and hippocampus of 6-month-old AD mice when compared to AD CON (Fig. 3C, E). WT mice showed no Aβ staining (not shown). Additionally, mouse cortical extracts were analyzed by Western blot for expression levels of APP and Aβ (Fig. 3F; 6E10), which showed that AD HFD mice had reduced cortical levels of APP (Fig. 3G) and Aβ (Fog. 3F), compared to AD CON.

Figure 3. HFD consumption starting at 1 month reduced Aβ brain pathology in 6-month-old AD mice.

A HFD reduced Aβ deposition throughout the brains of AD mice. % area covered in Aβ staining is indicated at each Bregma location. **B** HFD reduced Aβ deposition at multiple Bregma points in the brains of AD mice; each symbol represents a single Bregma point per mouse imaged. **C** Representative images of 6E10 staining used to visualize Aβ plaques in AD mice. Staining was quantified in the retrosplenial cortex (RSC), designated by boxed area, and hippocampus. WT mice had no detectable Aβ staining (not shown). **D, E** HFD reduced Aβ deposition in the RSC (**D**) and hippocampus (**E**) of AD mice. **F** Representative Western blots of cortical protein extracts isolated from 6-month-old WT and AD mice, showing expression of human Aβ and APP by 6E10 antibody. GAPDH was used for normalization. **G** HFD reduced levels of APP in cortical protein extracts isolated from AD mice. **H** HFD reduced levels of Aβ in cortical protein extracts isolated from AD mice. For **A** and **B**, n=5-8 mice per group. For **C-E**, n=6 mice per group. For **F-H**, n=7 per group. Results are from one representative experiment. Statistical analysis performed by Student's t test. *$p<0.05$, ****$p<0.0001$. Scale bar=500 μm.


A
Cohort I
Aβ (% area)
AD CON
AD HFD
1.18mm
0.98mm
0.50mm
0.26mm
0.02mm
-0.46mm
-0.94mm
-1.06mm
-1.34mm
-1.70mm
-1.94mm
-2.30mm
2.70mm
-2.92mm
3.28mm
B
Cohort I
****
Aβ (% area)
AD CON
AD HFD
C
AD CON
Bregma -2.92mm
Aβ
AD HFD
Bregma -2.92mm
Aβ
500 μm
500 μm
D
RSC (Cohort I)
Aβ (% area)
*
AD CON
AD HFD
E
Hippocampus (Cohort I)
Aβ (% area)
*
AD CON
AD HFD
F
WT CON
WT HFD
AD CON
AD HFD
WT CON
WT HFD
AD CON
AD HFD
WT CON
WT HFD
AD CON
AD HFD
WT CON
WT HFD
AD CON
AD HFD
APP
Aβ
GAPDH
G
APP (A.U.)
*
AD CON
AD HFD
H
Aβ (A.U.)
*
AD CON
AD HFD

Early HFD feeding improved cognitive function in 6-month-old AD mice.

Memory was assessed in 6-month-old AD mice by novel object recognition (NOR), which evaluates the ability of rodents to recognize a novel object in a familiar environment [78]. There was no difference in the total distance moved or velocity during training (Fig.4A, B) or testing (Fig.4C, D) between any groups, indicating that neither AD genotype nor HFD impaired locomotion. There were no differences between groups on the training day (Fig. 4E), indicating no place preference. As expected, AD CON mice spent less time exploring the novel object on the testing day compared to WT mice (Fig. 4F). However, AD HFD mice performed significantly better than AD CON and as well as WT groups (Fig. 4F).

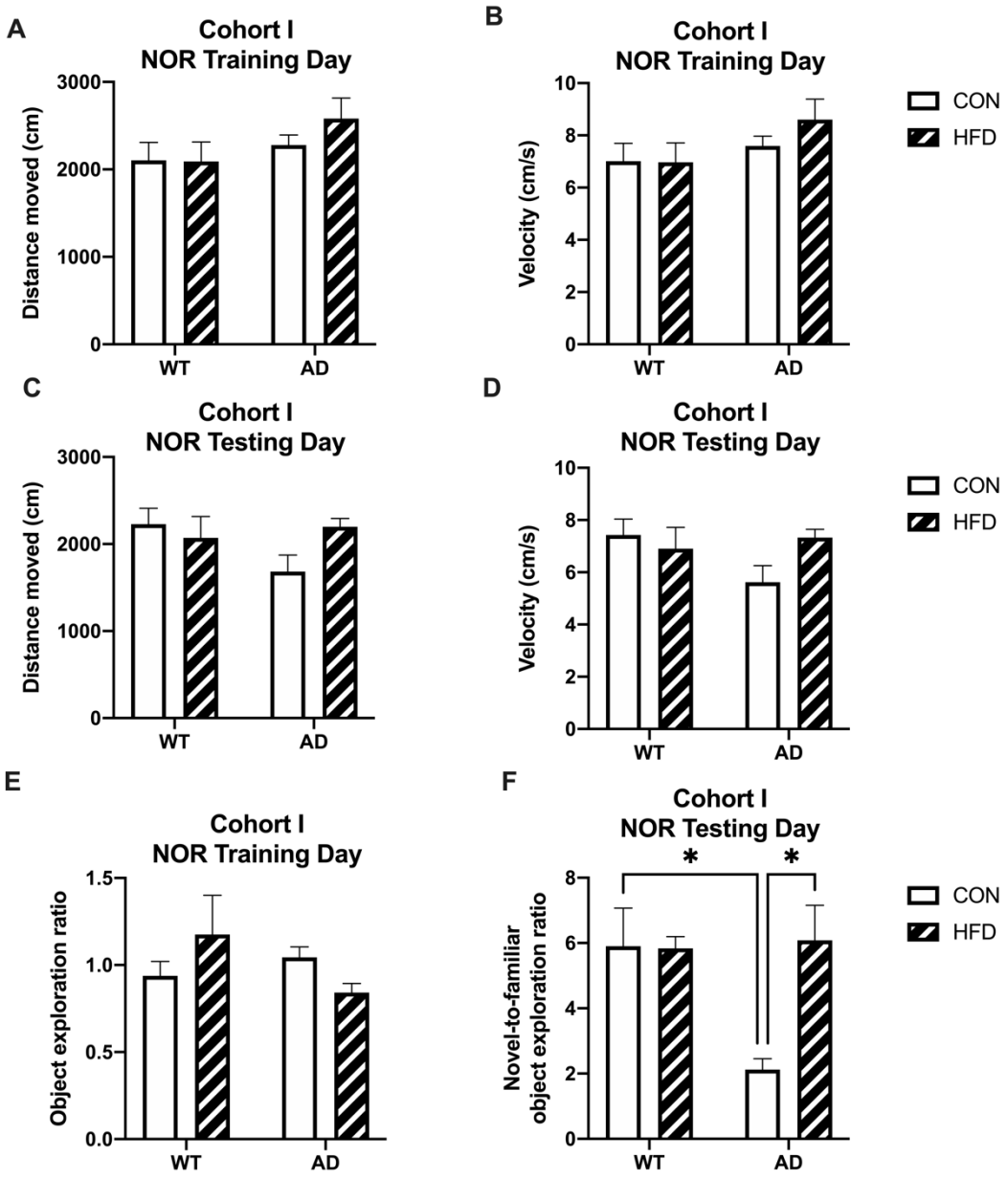


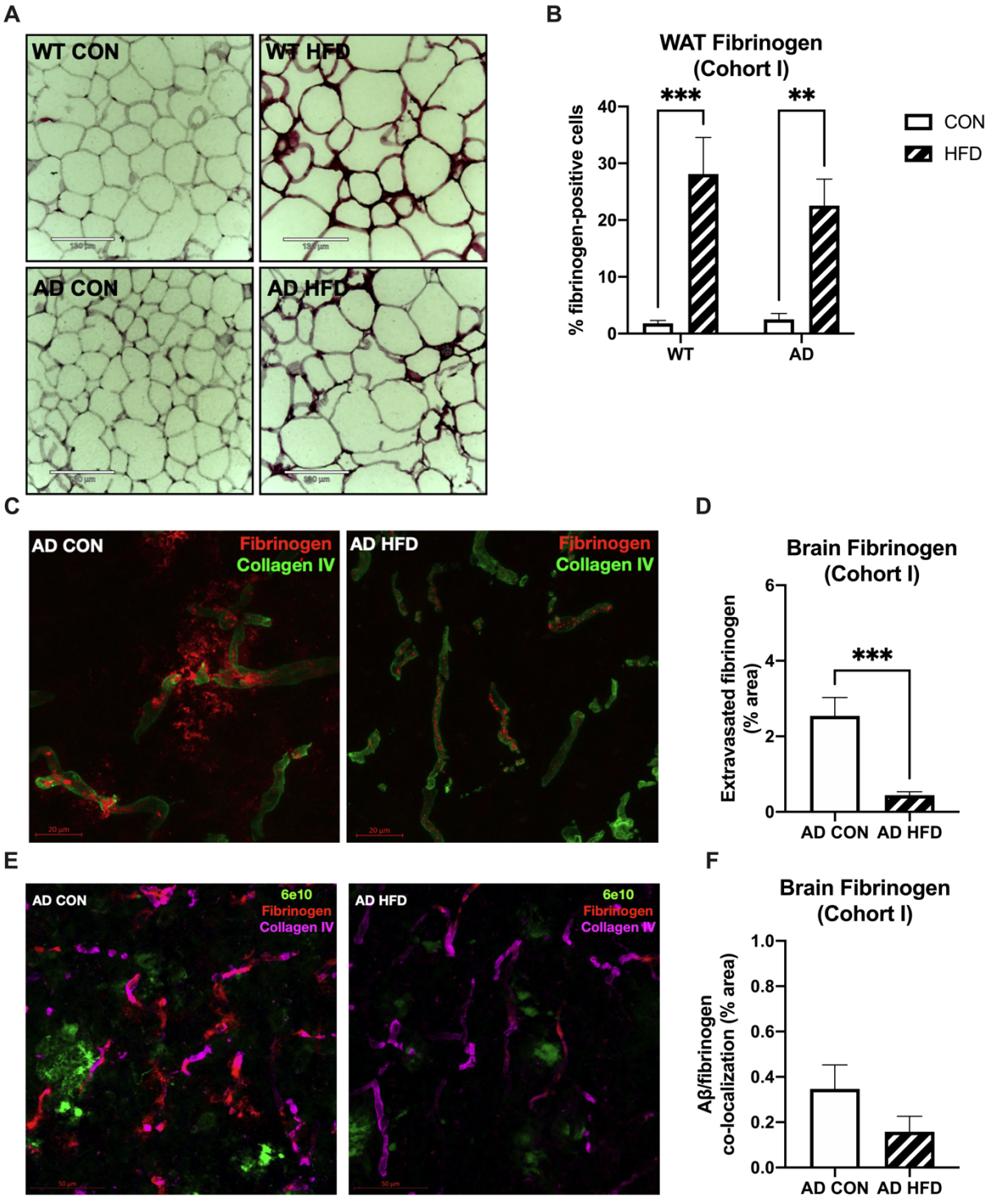
Figure 4. HFD consumption starting at 1 month improved cognition in 6-month-old AD mice. HFD did not affect the total distance moved or velocity during novel object recognition (NOR) training (**A, B**) or testing (**C, D**) days in WT or AD mice. **E** No differences between groups were present on the training day of NOR test, indicating no place preference. **F** HFD increased the novel-to-familiar object exploration ratio in AD mice on the testing day of the NOR test, indicating improved memory compared to AD CON. As expected, the object exploration ratio was lower in AD CON mice compared to WT groups. Results are from one representative experiment. Results are from one representative experiment. n=5-6 mice per group. Statistical analysis performed by two-way ANOVA. *$p<0.05$.

HFD consumption starting at 1 month-of-age affected fibrinogen extravasation into the brains of 6-month-old AD mice.

HFD induces fibrinogen deposition in white adipose tissue (WAT) of mice [79], so we investigated whether AD genotype affected this result. HFD significantly increased the percentage of fibrinogen-positive cells in both 6-month-old WT and AD mice (Fig. 5A, B), indicating that genotype did not affect fibrinogen deposition in WAT. Since AD animal models and human patients show deposition of fibrinogen in their brains [75], we also investigated for any change in this pathology after HFD feeding. While AD CON brains showed extensive fibrinogen extravasation from blood vessels into the brain parenchyma, AD HFD brains showed little extravasation (Fig. 5C, D). It has also been shown that Aβ and fibrinogen co-deposit in the brains of AD animal models and patients [80]. Upon investigation, we found minimal Aβ/fibrinogen co-deposition in the RSC of our experimental AD mice (Fig. 5E, F). While there was less Aβ/fibrinogen co-deposition in the AD HFD mouse brains than AD CON, this difference was not significant (Fig. 5E, F). Our findings showed that while dietary fats consumed at a young age did not affect fibrinogen deposition in WAT, it greatly protected the integrity of the BBB to prevent a blood protein from leaking into the brain tissue, which may aid in preventing Aβ/fibrinogen co-deposits.

Figure 5. HFD consumption starting at 1 month reduced fibrinogen extravasation into the RSC of 6-month-old mice.

A Representative images of white adipose tissue (WAT) fibrinogen staining. Scale bar=130 μm. **B** HFD increased percent fibrinogen-positive cells in WAT of WT and AD mice. Results are combined from two independent experiments. **C** Representative images of fibrinogen staining (red) show fibrinogen extravasation from collagen IV-positive blood vessels (green) into the RSC of AD CON, but not AD HFD mice. Scale bar=20 μm. **D** There was significantly less fibrinogen staining outside of blood vessels in RSC of AD HFD mice, indicating strong BBB integrity. **E** Representative triple-stained confocal microscopy images show Aβ deposits (green, 6E10) and fibrinogen extravasation (red) from collagen IV-positive blood vessels (purple) in the brain parenchyma of AD CON, but not AD HFD mice. Scale bar=50 μm. **F** HFD consumption caused a small, non-significant decrease in the level of Aβ/fibrinogen co-deposition in the RSC of AD mice. For **A** and **B**, n=9-10 mice per group, 3 sections per animal. Results are combined from two independent experiments. For **C** and **D**, n=6-7 mice per group, 3 sections per animal. Results are from one representative experiment. For **E** and **F**, n=8-9 mice per group, 3 sections per animal. Results are from one representative experiment. Statistical analysis performed by two-way ANOVA (**A** and **B**) or Student's t-test (**C** and **D**). **p<0.01, ***p<0.001.

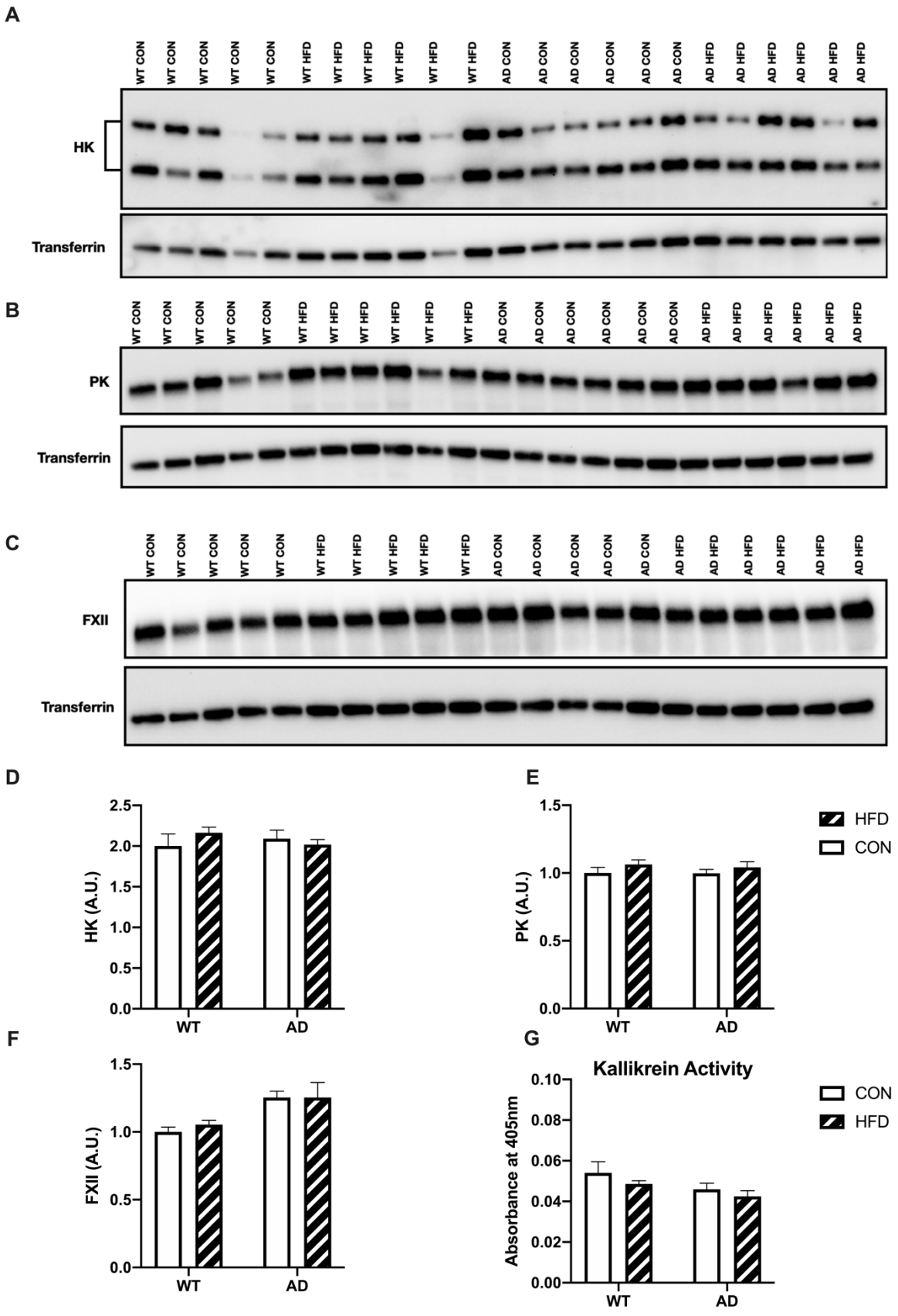

A
WT CON
WT HFD
AD CON
AD HFD
B
WAT Fibrinogen
(Cohort I)
***
**
% fibrinogen-positive cells
40
30
20
10
0
WT
AD
CON
HFD
C
AD CON
Fibrinogen
Collagen IV
AD HFD
Fibrinogen
Collagen IV
D
Brain Fibrinogen
(Cohort I)
Extravasated fibrinogen
(% area)
6
4
2
0
***
AD CON
AD HFD
E
AD CON
6e10
Fibrinogen
Collagen IV
AD HFD
6e10
Fibrinogen
Collagen IV
F
Brain Fibrinogen
(Cohort I)
Aβ/fibrinogen
co-localization (% area)
1.0
0.8
0.6
0.4
0.2
0.0
AD CON
AD HFD

HFD did not affect the activation of the plasma contact system in 6-month-old WT and AD mice.

The plasma contact system is comprised of a group of plasma proteins, including coagulation factor XII (FXII), prekallikrein (PK), and high molecular weight kininogen (HK), that can promote thrombin generation and fibrin clotting [81] as well as inflammation [82]. There is evidence supporting an overactivated contact system in AD pathogenesis [77, 83, 84]. Since both extravascular fibrin deposition and inflammation were decreased in AD mice after HFD consumption, we investigated whether dietary fats may have decreased contact system activation in the plasma of 6-month-old AD mice. We found that HFD did not affect the activation of HK (Fig. 6A, D), PK (Fig. 6B, E), or FXII (Fig. 6C, F), since we did not observe any group differences in the plasma levels of these proteins. We also used a chromogenic assay to assess the activity of plasma kallikrein. However, HFD did not affect kallikrein activity in the plasma of 6-month-old WT or AD mice (Fig. 6G).

Figure 6. HFD did not affect plasma contact system activation in 6-month-old WT and AD mice.

A-C Representative Western blots of plasma from 6-month-old WT and AD mice, examining contact system components. Transferrin was used for normalization. A HK expression. **B** PK expression. **C** FXII expression. **D-F** Quantification of protein expression from Western blot analyses. Plasma HK levels (**D**), plasma PK levels (**E**), and (**F**) plasma FXII levels were not affected by either AD genotype or HFD in Cohort I. G Neither AD genotype nor HFD affected kallikrein activity in the plasma of 6-month-old mice. For **A-F**, results are from three independent experiments, n=16-18 mice per group. For **G**, results are from one representative experiment, n=5-6 per group. Statistical analysis performed by two-way ANOVA.


A
WT CON
WT HFD
AD CON
AD HFD
HK
Transferrin
B
PK
Transferrin
C
FXII
Transferrin
D
HK (A.U.)
2.5
2.0
1.5
1.0
0.5
0.0
WT
AD
E
PK (A.U.)
HFD
CON
F
FXII (A.U.)
G
Kallikrein Activity
Absorbance at 405nm
0.10
0.08
0.06
0.04
0.02
0.00

HFD induced transcriptome changes in the cortex of 6-month-old AD mice.

To examine how HFD affected brain transcriptome changes, we fed 1-month-old WT and AD mice a HFD or CON for 20 weeks. At 6 months, we extracted RNA from the cortices of HFD- and CON-fed mice and performed RNAseq. First, we assessed sample similarity by principal component analysis (PCA), where the greatest sources of variation are reduced to principal components and the separation of samples along these principal components is evaluated. PCA showed that replicates within each experimental group clustered together and that our experimental conditions (WT vs AD genotype, CON vs HFD) represented the major source of variation in the data (Fig. 7). Therefore, we proceeded with differential gene expression analysis of all samples. In WT mice, HFD affected the expression of 7 genes, with 4 genes upregulated and 3 genes downregulated in HFD-fed vs CON-fed WT mice (Fig. 8A). However, in AD mice, the number of differentially expressed genes was much higher with a total of 43 genes affected by HFD consumption (Fig. 8B). Specifically, we detected 30 upregulated genes and 13 downregulated genes in HFD-fed vs CON-fed AD mice (Fig. 8B). However, the interaction between the genotype and the diet seemed to induce dramatically different effects on the transcriptome. In CON-fed mice, we detected a total of 226 differentially expressed genes with 208 genes that were upregulated and 18 genes that were downregulated in AD vs WT mice (Fig. 9A). HFD consumption increased the number of differentially expressed genes, as we found a total of 345 differentially expressed genes with 292 genes upregulated and 53 genes downregulated in HFD-fed AD mice vs HFD-fed WT mice (Fig. 9B). Next, we determined which gene ontology (GO) categories were affected by CON vs HFD in AD mice. In AD CON vs AD HFD mice, the largest proportion of genes had a significant change to gene expression in the following categories: positive regulation of protein folding, regulation of protein folding, protein folding, chaperone-mediated protein folding, cell redox homeostasis, response to nutrient, response to endoplasmic reticulum stress, microtubule cytoskeleton organization, cytoskeleton organization, and cellular response to stress (Fig. 10) Genes upregulated by HFD in AD mouse cortex that were included in the "response to stress" GO category included *Cryab, Pdia4, Chordc1, Pdia3, Dnajc3, Dnajb11, Hsp90b1, Calr,* and *Fkbp4* (Fig. 11). Genes in the "cellular response to stress" GO category that were upregulated by HFD in AD mice included *Sdf2l1, Hyou1*, and *Hnrnpm*, while *Rhob* and *Ackr3* were downregulated (Fig. 12). Finally, we quantified the expression of human *APP*, human *PSEN1*, mouse *App*, and mouse *Psen1* in the cortex of HFD-fed WT and AD mice to determine whether HFD induced changes in APP processing. While HFD did not affect the expression of human *PSEN1*, mouse *App*, or mouse *Psen1* in AD mice, HFD significantly reduced the expression of human *APP* (Fig. 13).

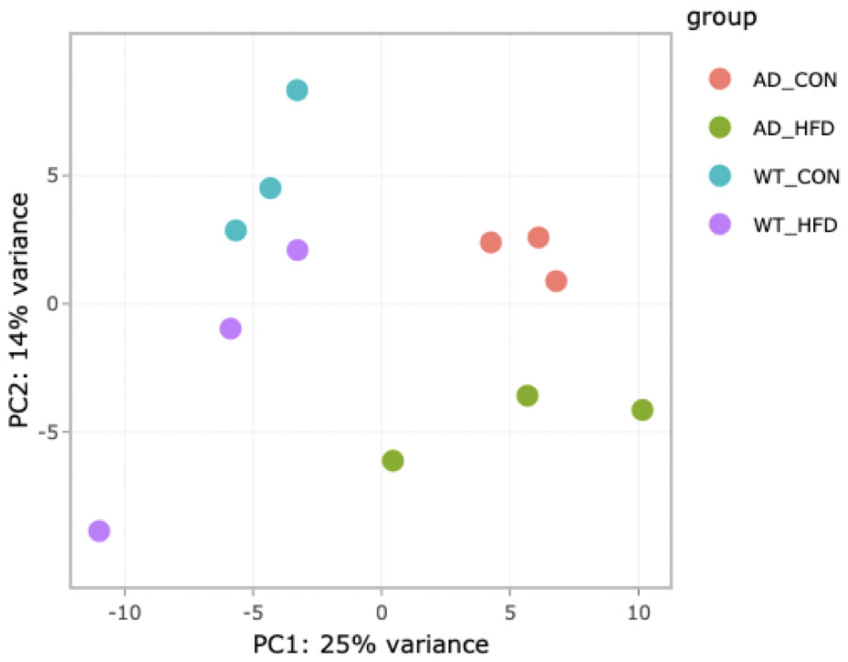


Figure 7. Differential gene expression analysis showed high sample similarity within each group.
Principal component analysis of RNAseq samples isolated from the cortex of AD CON (pink circles), AD HFD (green circles), WT CON (blue circles), and WT HFD (violet circles) mice. n=3 mice per group. Results are from one representative experiment.

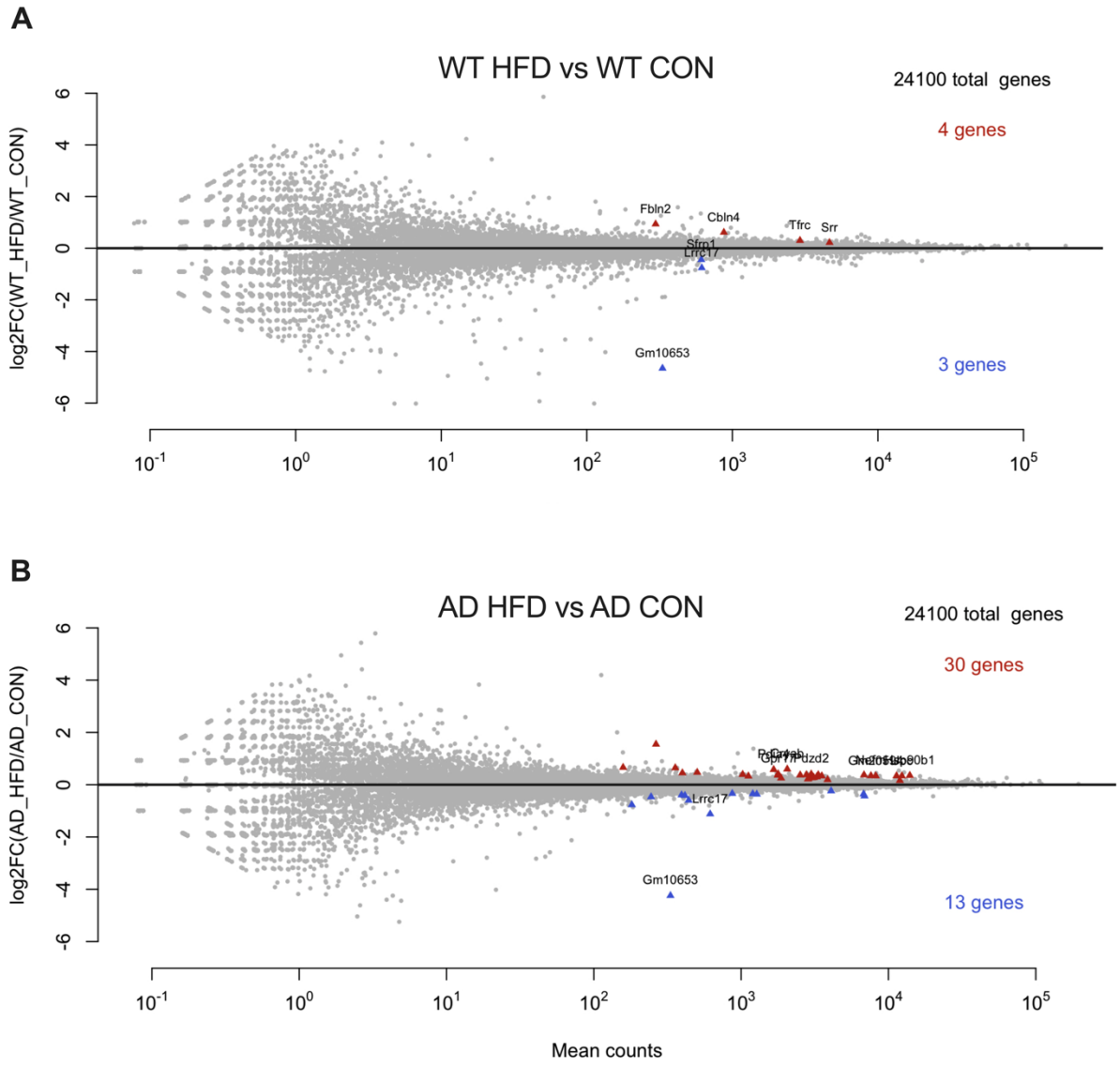


Figure 8. High fat diet affected the differential expression of 7 genes in WT and 43 genes in AD mice.

MA-plots for differential expression analysis in RNAseq samples isolated from the cortex of WT CON and WT HFD mice (**A**) or AD CON and AD HFD mice (**B**). Red color indicates significantly upregulated genes, and blue color indicates significantly downregulated genes. n=3 mice per group. Results are from one representative experiment.

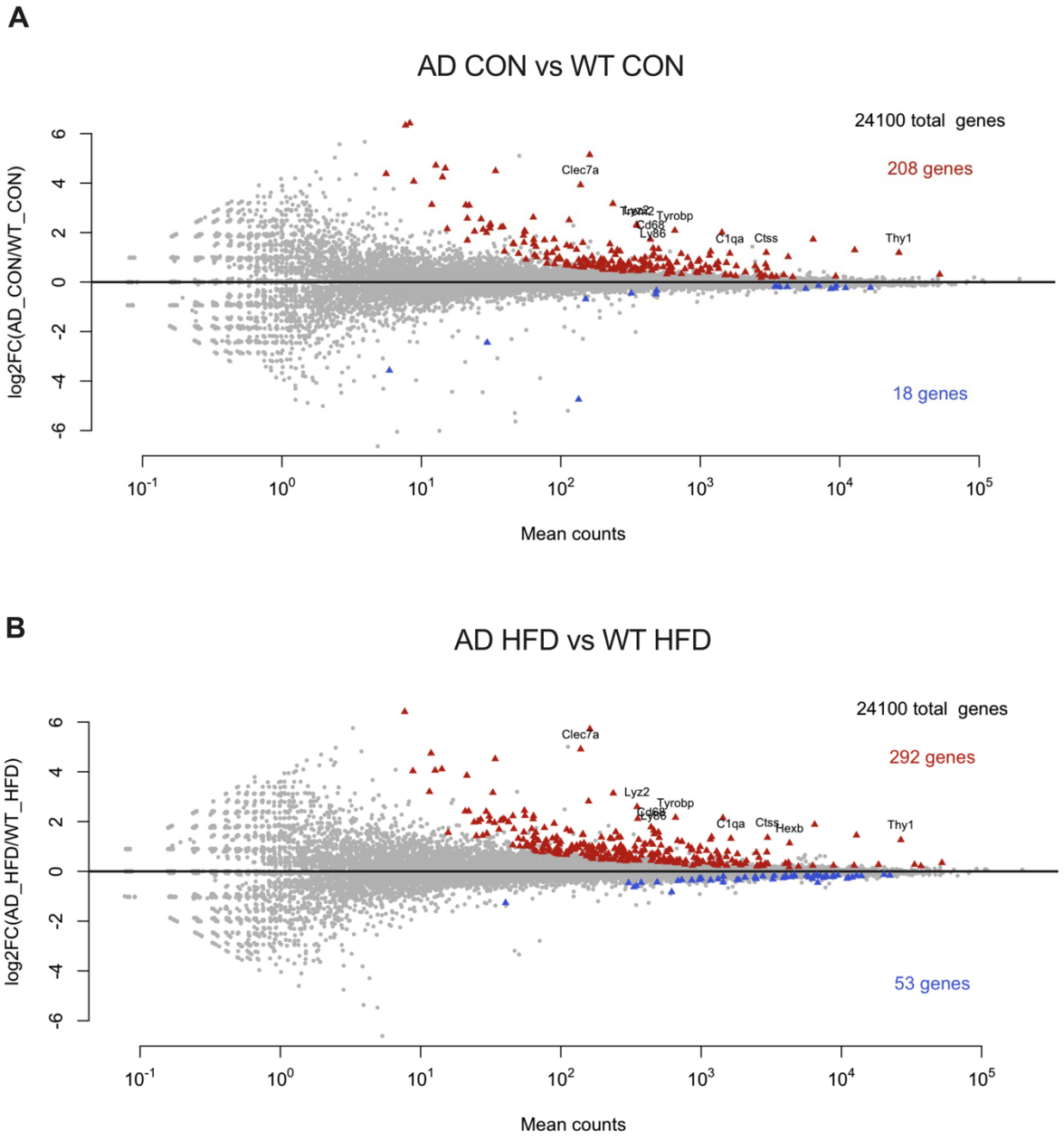


Figure 9. AD genotype affected the differential expression of 226 genes in CON-fed and 354 genes in HFD-fed mice.
MA-plots for differential expression analysis in RNAseq samples isolated from the cortex of WT CON and AD CON mice (**A**) or WT HFD and AD HFD mice (**B**). Red color indicates significantly upregulated genes, blue color indicates significantly downregulated genes. n=3 mice per group. Results are from one representative experiment.

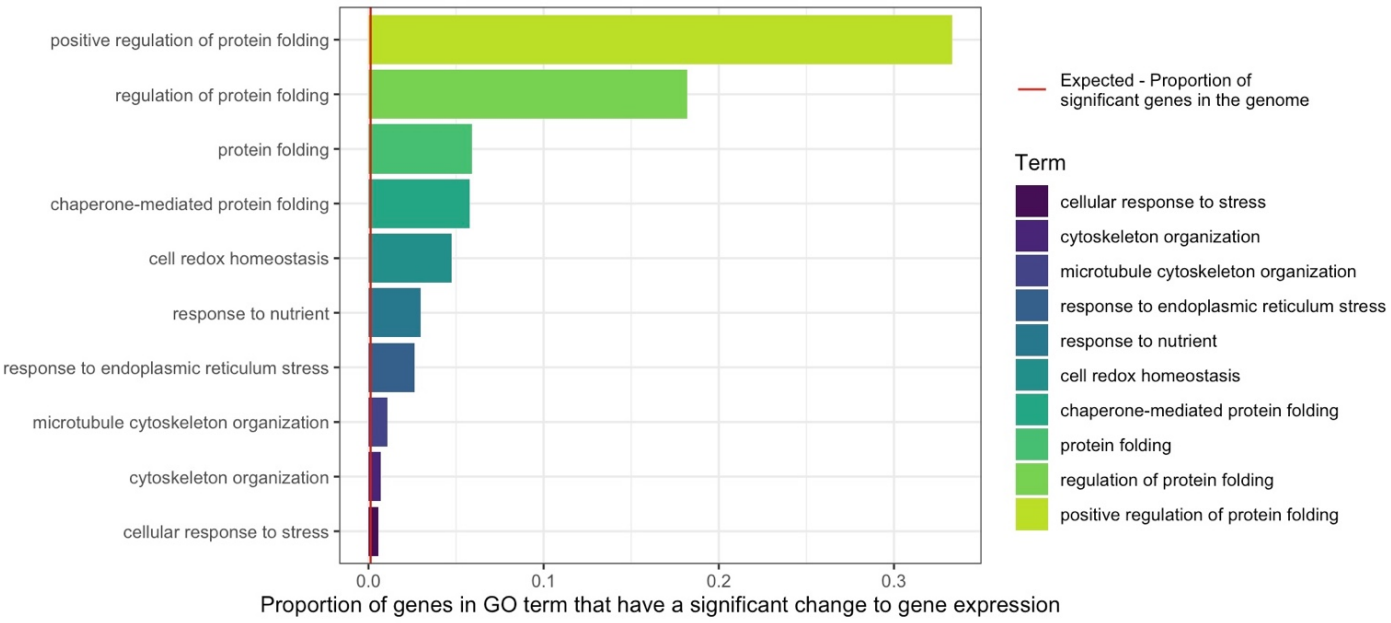


Figure 10. Gene ontology (GO) analysis revealed the upregulation of genes in several GO categories in AD HFD vs. AD CON mice.

GO term enrichment analysis of upregulated genes. The vertical coordinates are the enriched GO terms, and the horizontal coordinates are the proportion of upregulated gene with a significant change to gene expression in these GO terms. n=3 mice per group. Results are from one representative experiment.

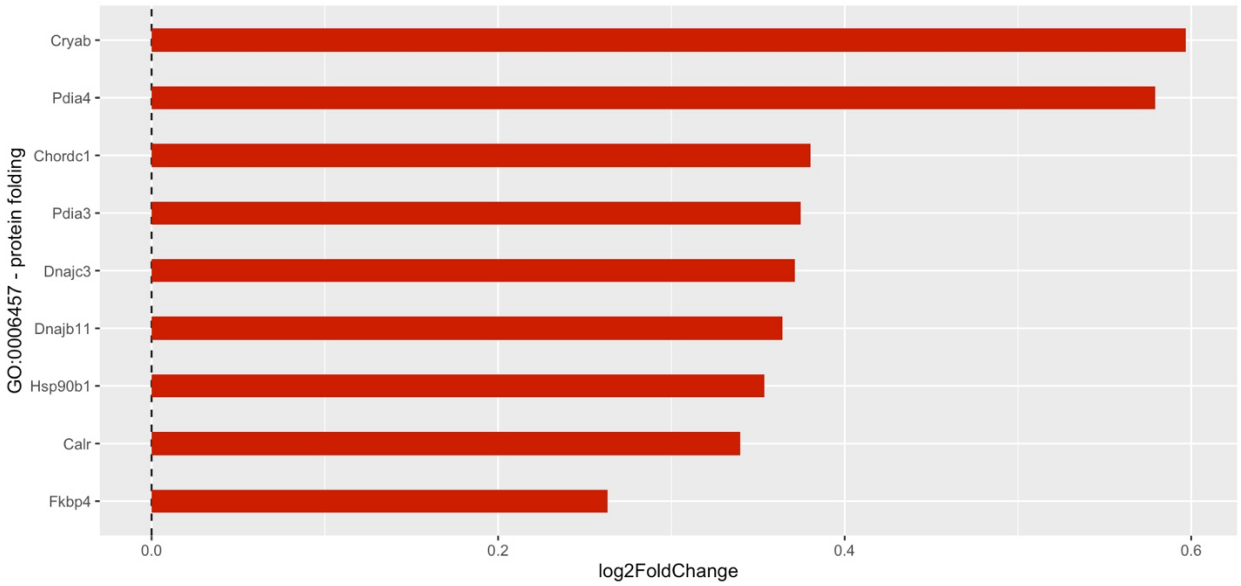


Figure 11. Protein folding genes that are upregulated AD mice upon HFD consumption. GO term enrichment analysis of differentially expressed genes within the "protein folding" category. Red color indicates significantly upregulated genes. n=3 mice per group. Results are from one representative experiment.

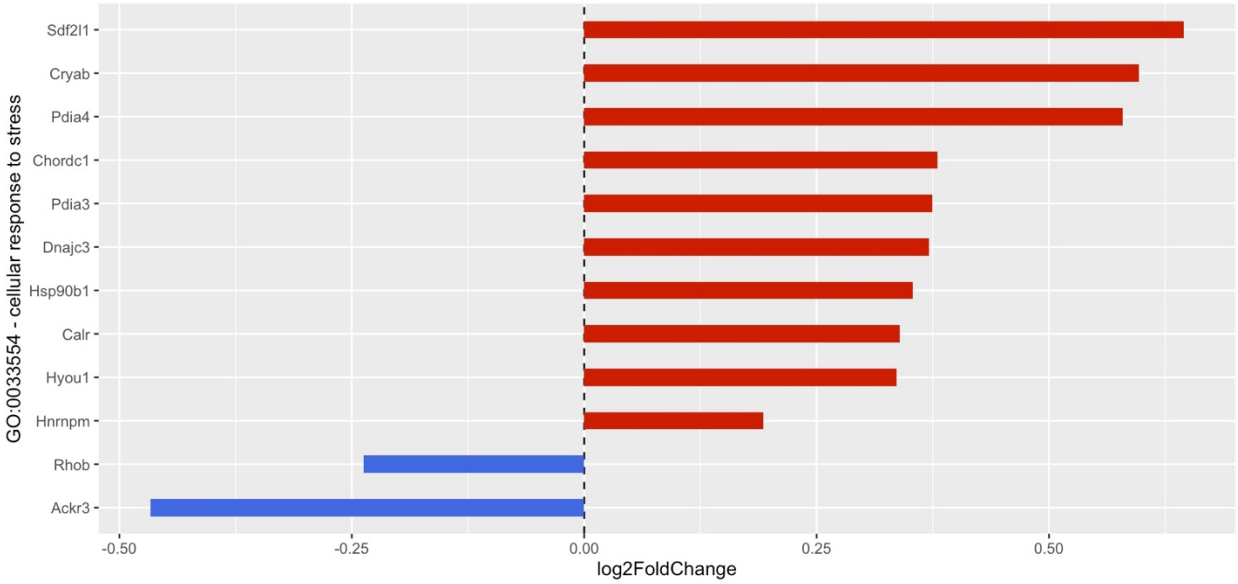


Figure 12. Genes associated with cellular response to stress that are differentially regulated in AD mice fed a HFD.

GO term enrichment analysis of differentially expressed genes within the "Cellular response to stress" category. Red color indicates significantly upregulated genes. Blue color indicates significantly downregulated genes. n=3 mice per group. Results are from one representative experiment.

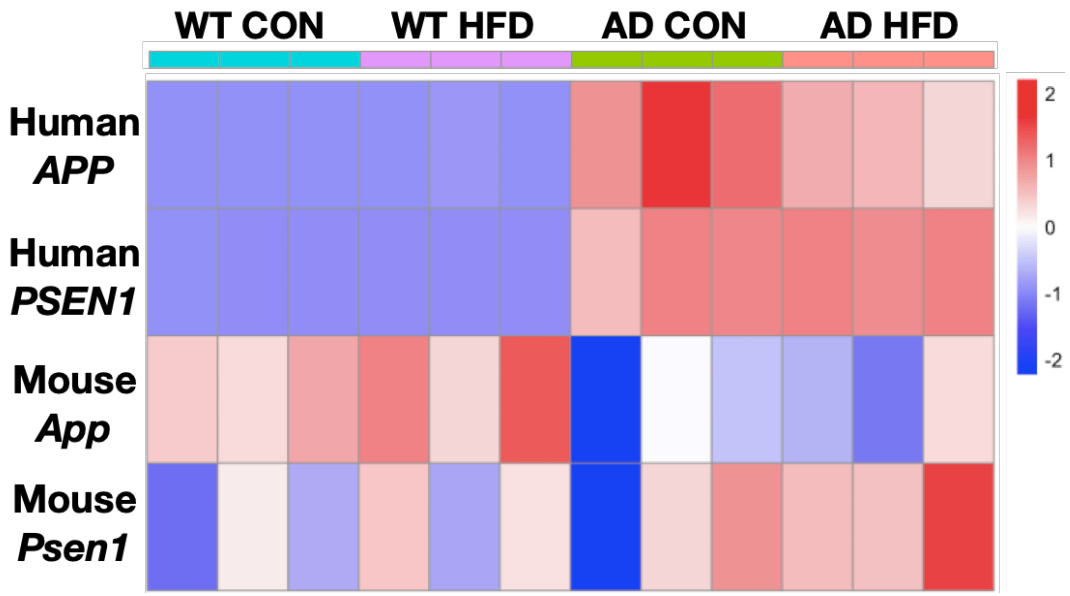


Figure 13. HFD reduced the expression of human APP gene in AD mice.
RNAseq heatmap shows human *APP*, human *PSEN1*, mouse *App*, and mouse *Psen1* expression in the cortex of WT CON, WT HFD, AD CON, and AD HFD mice. The color scale bar shows z-score values after z-score row normalization. n=3 mice per group. Results are from one representative experiment.

Chapter 4: The Effect of Delayed Dietary Fat Supplementation on AD-related Pathology and Cognitive Function

HFD consumption starting at 3 months induced weight gain and affected body composition in 8-month-old mice.

To investigate how a delayed onset of HFD consumption affects AD-related pathology in mice, we placed 3-month-old mice (Cohort II) on a diet of 60% fat HFD or CON for 20 weeks until the mice were 8 months-of-age (Fig. 14A). HFD induced significant weight gain in both WT and AD mice, despite the delayed onset of HFD consumption (Fig. 14B, C). HFD also increased percent body fat mass and decreased percent lean mass in 8-month-old HFD-fed WT and AD mice (Fig. 14D, E). HFD impaired the ability to clear circulating glucose after a GTT in both WT and AD mice, when compared to CON-fed mice, thus inducing glucose intolerance (Fig. 15A, B). Additionally, HFD induced hyperglycemia after a period of fasting in 8-month-old WT and AD mice (Fig. 15C). Fasting also induced an increase in blood ketones in 8-month-old HFD-fed WT as well as AD mice (Fig. 15D). Additionally, after refeeding, both WT HFD and AD HFD mice showed an increase in blood ketones when compared to CON-fed mice (Fig. 15E).

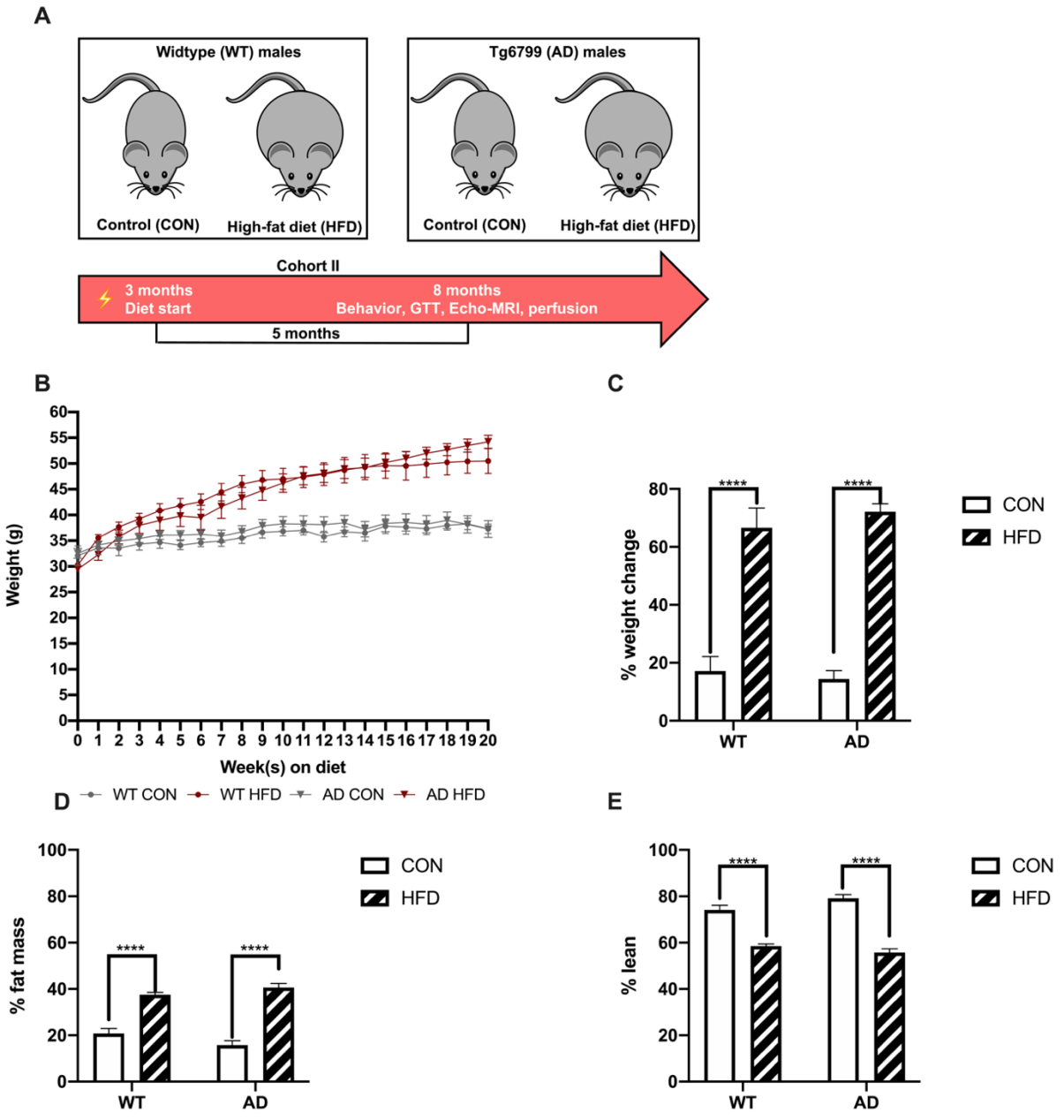


Figure 14. Delayed HFD consumption caused significant weight gain and affected body composition similarly in 8-month-old WT and AD mice.

A Schematic shows feeding timeline in WT and AD mice in Cohort II. The lightning icon indicates the onset of AD-related pathology. **B** Male mice (3 months old) fed a HFD vs CON had greater weight gain. **C** HFD induced a significant increase in % weight change in both WT and AD mice, when comparted to CON-fed mice. **D** HFD increased % fat mass in 8-month-old WT and AD mice. **E** HFD decreased % lean mass in 8-month-old WT and AD mice. n=5-9 mice per group. Results are from one representative experiment. Statistical analysis performed by two-way ANOVA. ****p<0.0001.

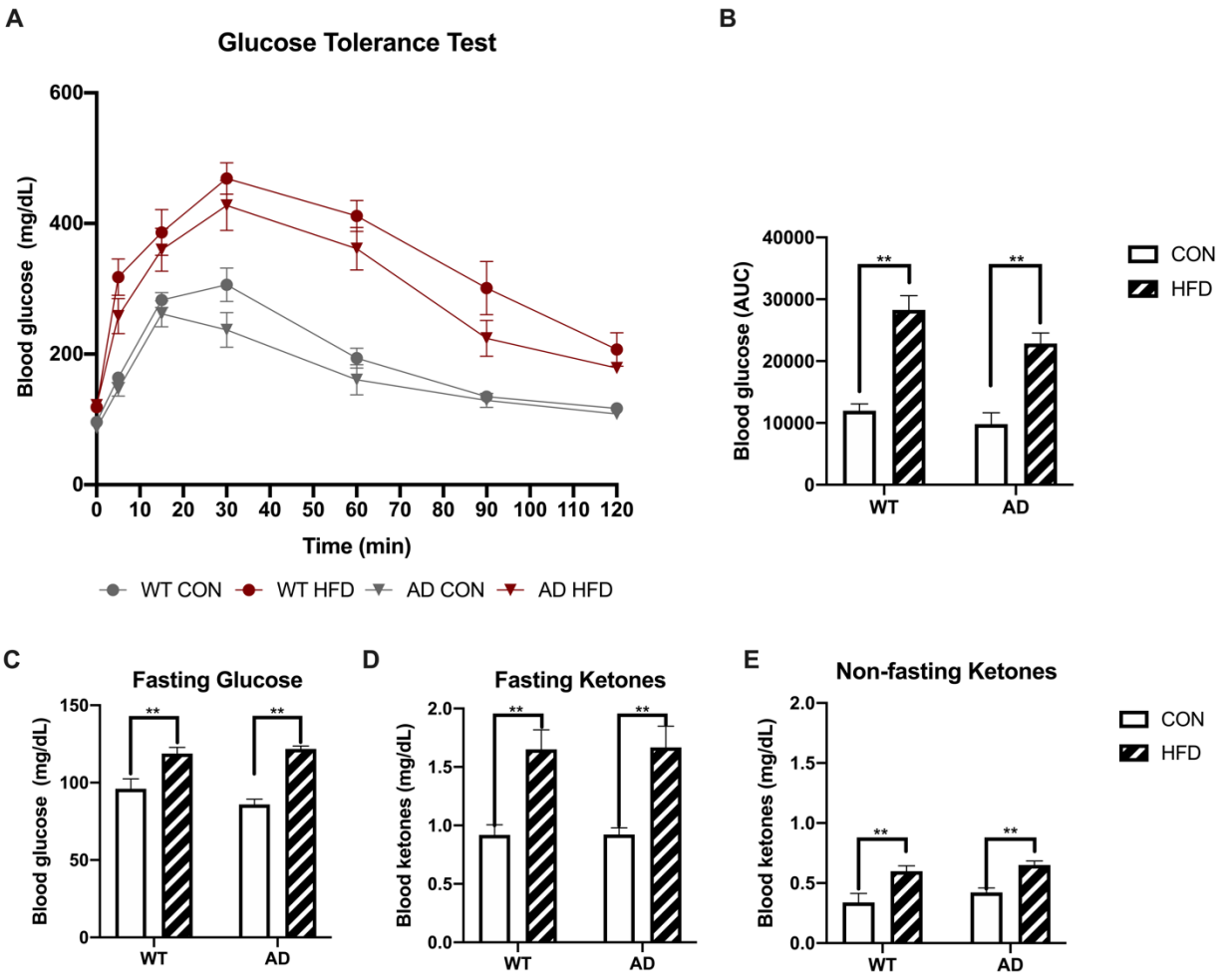


Figure 15. HFD induced hyperglycemia, affected glucose metabolism, and caused ketogenesis in 8-month-old mice.

A HFD impaired glucose clearance in WT and AD mice after a glucose tolerance test (GTT). **B** HFD-fed WT and AD mice showed higher blood glucose levels after GTT, compared to CON-fed mice, as indicated by area under the curve (AUC). **C** HFD increased fasting blood glucose levels in WT and AD mice. **D** HFD increased fasting blood ketone levels in WT and AD mice. **E** HFD increased non-fasting blood ketones in both WT and AD mice. **F** HFD increased the level of plasma cholesterol in WT and AD mice. **G, H** HFD induced a small, non-significant increase in plasma HDL levels but not plasma LDL levels in WT and AD mice. For **A**-**C**, **F**-**H**: n=8-11 mice/group. Results are from one representative experiment. For **D**, **E**: n=5-9 mice/group. Results are from one representative experiment. Statistical analysis performed by two-way ANOVA. **p<0.01.

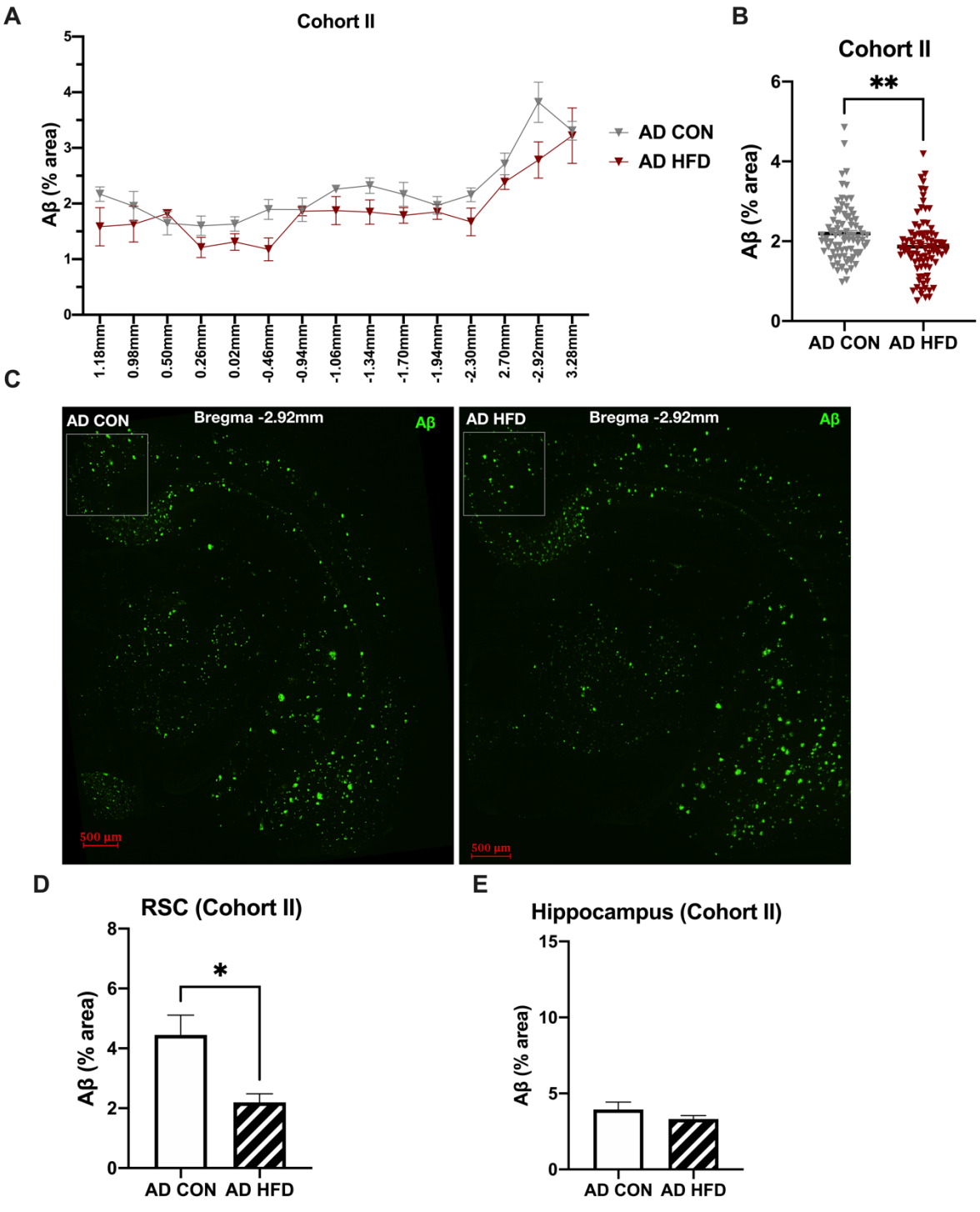
HFD consumption starting at 3 months reduced Aβ pathology and improved cognitive function in 8-month-old AD mice.

In Tg6799 mice, Aβ plaque deposition starts as early as at 2 months-of-age. By 6 months-of-age, Tg6799 mice display abundant parenchymal Aβ plaque deposition and memory deficits [30]. We investigated whether the timing of HFD feeding was related to the improvement in AD-related pathology and cognition in our AD mouse cohorts. Therefore, we delayed the onset of HFD administration until 3 months-of-age, after AD-related pathology begins to develop. The diet regimen continued until Cohort II mice were 8 months old.

In Cohort II, HFD consumption starting at 3 months-of-age significantly reduced Aβ plaque burden across the entire brain (Fig. 16A, B). More specifically, there was significantly less Aβ staining in the RSC of AD HFD vs AD CON mice (Fig. 16C, D), yet no difference was detected in the hippocampus (Fig. 16C, E). Neither genotype nor diet affected locomotion on NOR training day (Fig. 17A, B). However, AD CON mice showed an increase in total distance moved and velocity compared to WT CON mice during NOR testing (Fig. 17C, D), indicating that AD genotype caused hyperactivity in 8-month-old mice. Interestingly, HFD consumption corrected this phenotype, restoring locomotion to WT levels in Cohort II AD mice (Fig. 17C, D). There were no differences between groups in object exploration on the training day (Fig. 17E). However, during testing, HFD increased the novel-to-familiar object exploration ratio in AD mice when compared to AD CON mice (Fig. 17F), indicating that HFD consumption starting at 3 months, after the onset of AD-like pathology, still led to improved cognitive function in 8-month-old AD mice. HFD had a similar effect on AD-related pathology and cognitive function in 6-month-old and 8-month-old mice, despite the delayed onset of high-fat feeding (1 month vs 3 months). Therefore, consuming dietary fats prior to significant AD pathology may be protective and delay disease progression.

Figure 16. HFD consumption starting at 3 months reduced Aβ pathology in the RSC of AD mice.

A HFD consumption starting at 3 months and lasting until 8 months (Cohort II) reduced Aβ deposition throughout the brains of 8-month-old AD mice. **B** Quantification of Aβ staining throughout the brains of Cohort I 8-month-old AD mice showed a decrease in overall Aβ pathology. Each symbol represents a single Bregma point. **C** Representative images of Aβ staining in whole brain slices of 8-month-old AD mice. Staining was quantified in the RSC, designated by boxed area, and hippocampus. WT mice had no detectable Aβ deposits (not shown). Scale bar=500 μm. **D, E** In Cohort II, delaying the onset of HFD to 3 months-of-age reduced Aβ deposition in the RSC but not the hippocampus of 8-month-old AD mice.


A
Cohort II
Aβ (% area)
AD CON
AD HFD
1.18mm
0.98mm
0.50mm
0.26mm
0.02mm
-0.46mm
-0.94mm
-1.06mm
-1.34mm
-1.70mm
-1.94mm
-2.30mm
2.70mm
-2.92mm
3.28mm
B
Cohort II
**
Aβ (% area)
AD CON
AD HFD
C
AD CON
Bregma -2.92mm
Aβ
500 µm
AD HFD
Bregma -2.92mm
Aβ
500 µm
D
RSC (Cohort II)
*
Aβ (% area)
AD CON
AD HFD
E
Hippocampus (Cohort II)
Aβ (% area)
AD CON
AD HFD

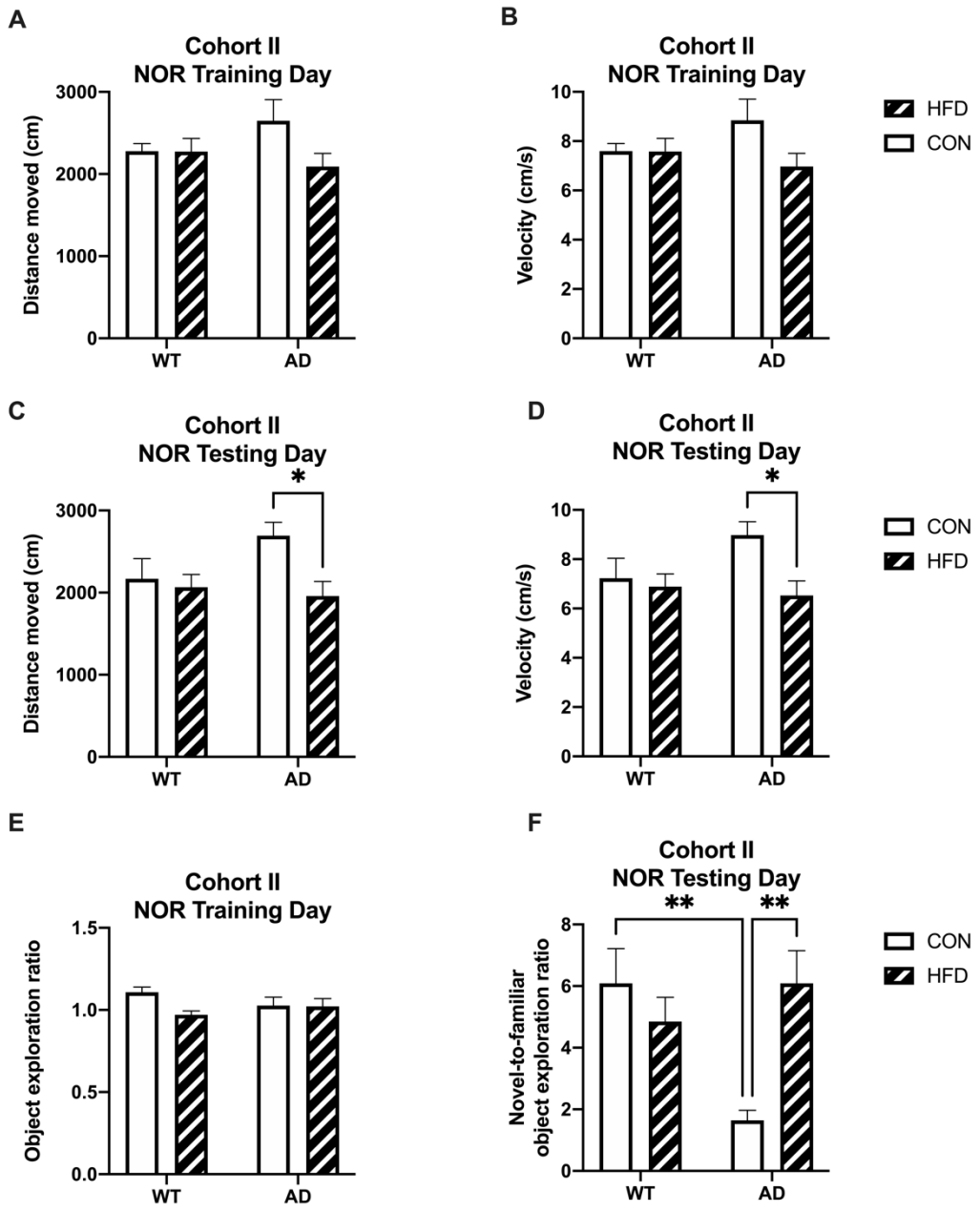


Figure 17. HFD consumption starting at 3 months improved cognition in 8-month-old AD mice.

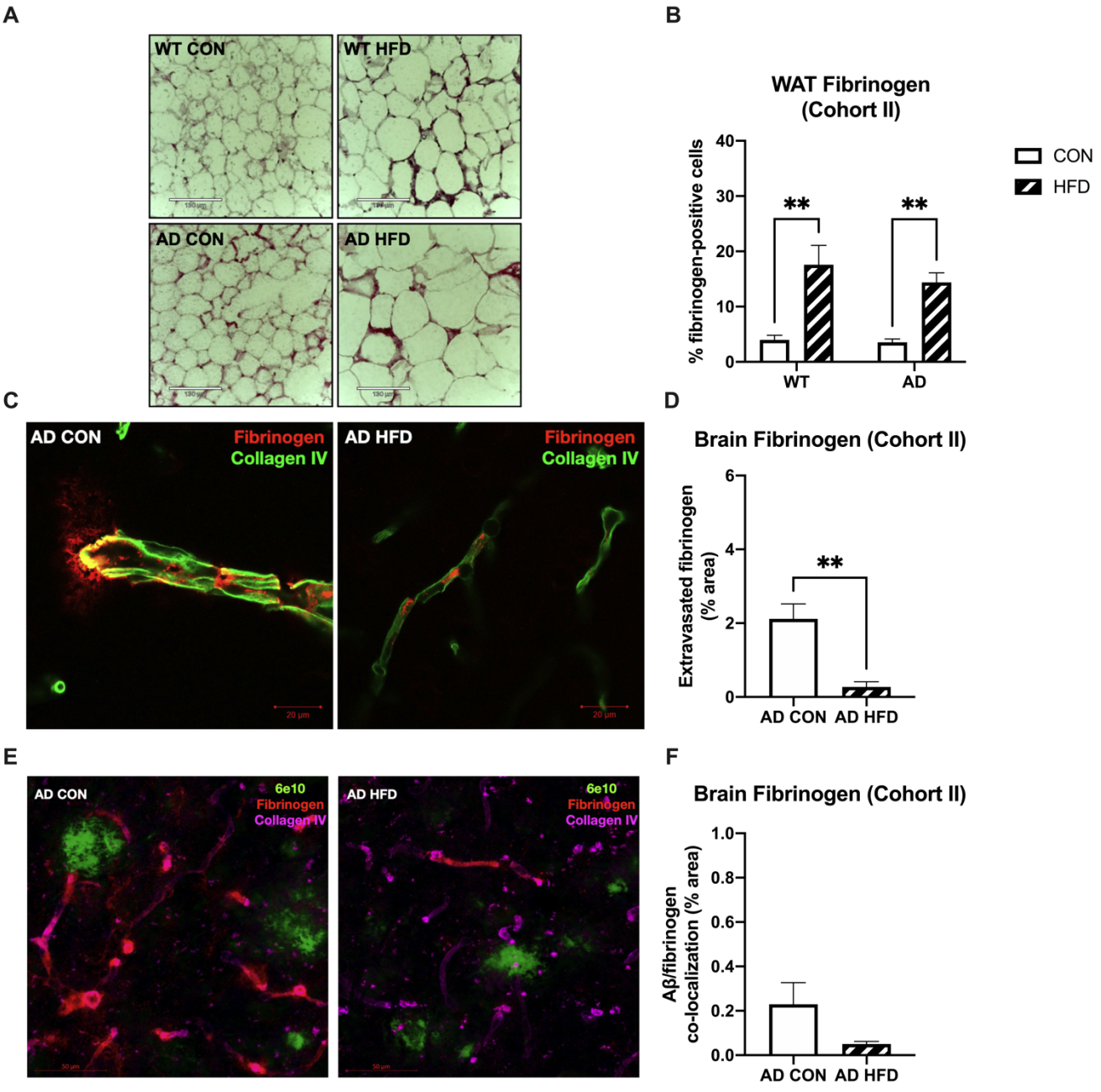
HFD did not affect the total distance moved or velocity during novel object recognition (NOR) training (**A, B**). HFD reduced the total distance moved and velocity in AD mice during NOR testing (**C, D**). **E** No differences in the object exploration ratio were present between groups on the training day of NOR test, indicating no place preference. **F** HFD increased the novel-to-familiar object exploration ratio in AD mice on the testing day of the NOR test, indicating improved memory compared to AD CON. The object exploration ratio was lower in AD CON mice compared to WT groups. Results are from one representative experiment. n=5-9 mice per group. Statistical analysis performed by two-way ANOVA. *p<0.05. **p<0.01.

HFD consumption starting at 3 months reduced fibrinogen extravasation into the brains of AD mice.

In Cohort II, where the 20-week HFD regimen was delayed until mice were 3 months old, HFD significantly increased the percentage of fibrinogen-positive cells in the WAT of both WT and AD mice (Fig. 18A, B). In parallel with our findings in 6-month-old AD mice, HFD also significantly reduced fibrinogen extravasation from blood vessels into the brain parenchyma of 8-month-old AD mice in this cohort (Fig. 18C, D). Therefore, despite the delayed onset of feeding, HFD helped improve BBB integrity. HFD did not affect Aβ/fibrinogen co-deposition in the RSC of 8-month-old AD mice, although we observed a small non-significant decrease in Aβ/fibrinogen co-localization in AD HFD mice (Fig. 18E, F).

Figure 18. HFD consumption starting at 3 months reduced fibrinogen extravasation into the RSC of 8-month-old mice.

A Representative images of WAT fibrinogen staining in 8-month-old mice after 20 weeks of experimental diet. **B** HFD increased percentage of fibrinogen-positive cells in WAT of 8-month-old WT and AD mice. **C** Representative images of fibrinogen staining (red) show fibrinogen extravasation from collagen IV-positive blood vessels (green) into the RSC of 8-month-old AD CON mice, but not AD HFD mice. **D** HFD significantly reduced fibrinogen staining outside of blood vessels in RSC of 8-month-old AD mice. **E** Representative triple-stained images of Aβ deposits (green, 6E10) and fibrinogen extravasation (red) from collagen IV-positive blood vessels (purple) in the brain parenchyma of 8-month-old AD CON, but not AD HFD mice. **F** HFD had a small but insignificant effect on the interaction between Aβ and fibrinogen in the RSC of 8-month-old AD mice. For **A** and **B**, n=5-6 mice per group, 3 slices per animal. For **C** and **D**, n=7 mice per group, 3 slices per animal. For **E** and **F**, n=4-5 mice per group, 3 slices per animal. Results are from one representative experiment. Statistical analysis performed by two-way ANOVA. **p<0.01. Scale bar=130μm in **A**; 20 μm in **C;** 20 μm in **E**.


A
WT CON
WT HFD
AD CON
AD HFD
B
WAT Fibrinogen
(Cohort II)
% fibrinogen-positive cells
40
30
20
10
0
**
**
WT
AD
CON
HFD
C
AD CON
Fibrinogen
Collagen IV
AD HFD
Fibrinogen
Collagen IV
20 µm
20 µm
D
Brain Fibrinogen (Cohort II)
Extravasated fibrinogen
(% area)
6
4
2
0
**
AD CON
AD HFD
E
AD CON
6e10
Fibrinogen
Collagen IV
AD HFD
6e10
Fibrinogen
Collagen IV
F
Brain Fibrinogen (Cohort II)
Aβ/fibrinogen
co-localization (% area)
1.0
0.8
0.6
0.4
0.2
0.0
AD CON
AD HFD

Chapter 5: The Effect of Late Dietary Fat Supplementation on AD-related Pathology and Cognitive Function

HFD consumption starting at 6 months induced weight gain and affected body composition in 11-month-old mice.

To investigate how a delayed onset of HFD consumption affects AD-related pathology in mice after the onset of amyloidosis and cognitive decline, we placed 6-month-old mice (Cohort III) on a diet of 60% fat HFD or CON for 20 weeks until the mice were 11 months-of-age (Fig. 19A). Despite the delayed onset of HFD consumption, HFD induced small, but significant weight gain in both WT and AD mice (Fig. 19B, C). Interestingly, CON-fed AD mice showed a decrease in the % weight change, indicating that weight loss accompanied aging in 11-month-old AD mice fed a CON (Fig. 19B, C). HFD also increased percent body fat mass and decreased percent lean mass in 11-month-old HFD-fed AD, but not WT mice (Fig. 19D, E). HFD impaired the ability to clear circulating glucose after a GTT in AD, but not WT mice, thus inducing glucose intolerance in HFD-fed AD mice only (Fig. 20A, B). However, HFD induced hyperglycemia after a period of fasting in both WT and AD mice at 11 months-of-age (Fig. 20C). We did not detect any group differences in either fasting or non-fasting blood ketone levels (Fig. 20D, E).

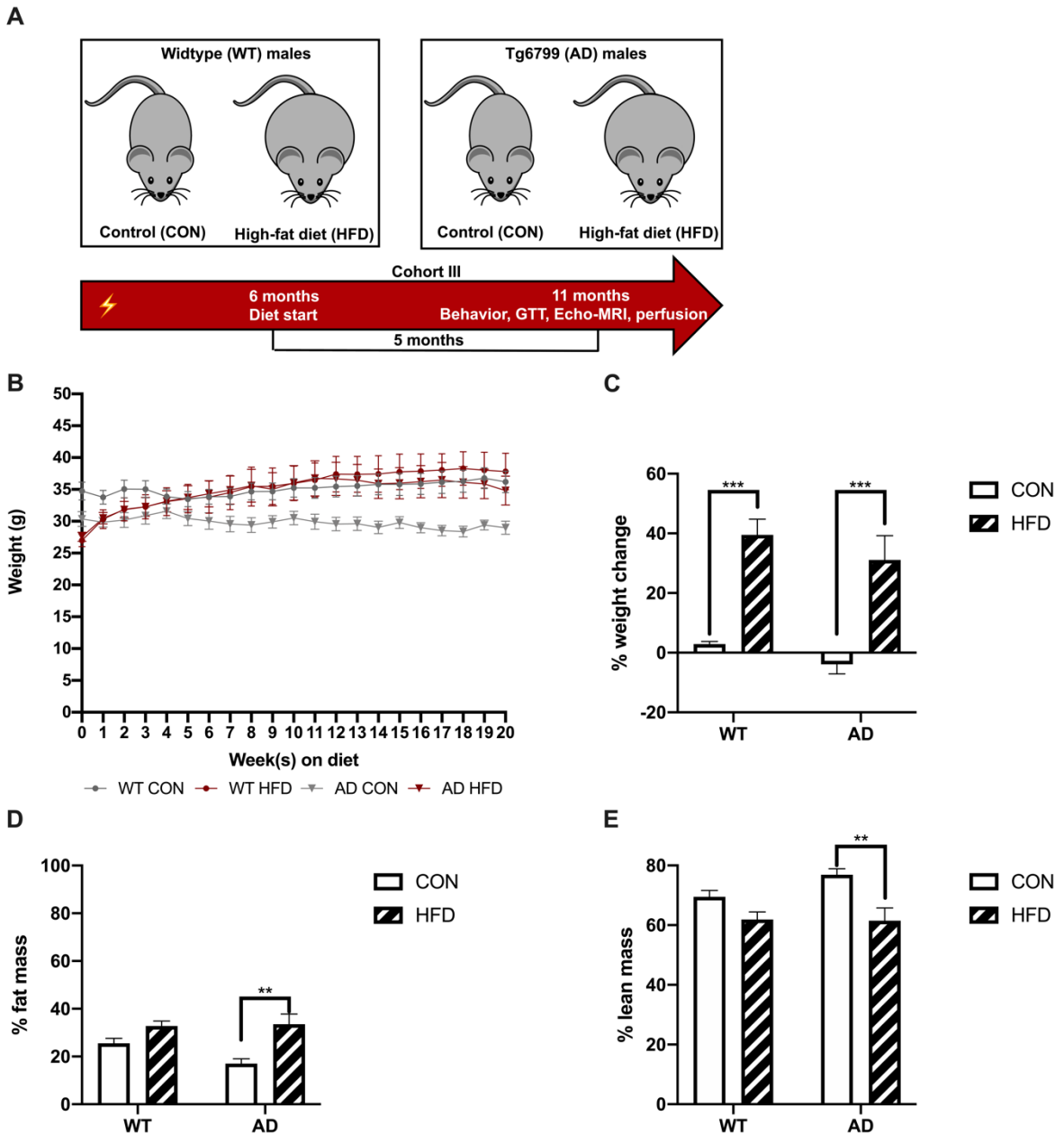


Figure 19. Delayed HFD consumption caused significant weight gain and affected body composition similarly in 11-month-old WT and AD mice.

A Schematic shows feeding timeline in WT and AD mice in Cohort III. The lightning icon indicates the onset of AD-related pathology. **B** Male mice (6 months old) fed a HFD vs CON had greater weight gain. **C** HFD induced a significant increase in % weight change in both WT and AD mice, when comparted to CON-fed mice. **D** HFD increased % fat mass in 11-month-old AD, but not WT mice. **E** HFD decreased % lean mass in 11-month-old AD, but not WT mice. n=5-9 mice per group. Results are from one representative experiment. Statistical analysis performed by two-way ANOVA. .**p<0.01. ***p<0.001.

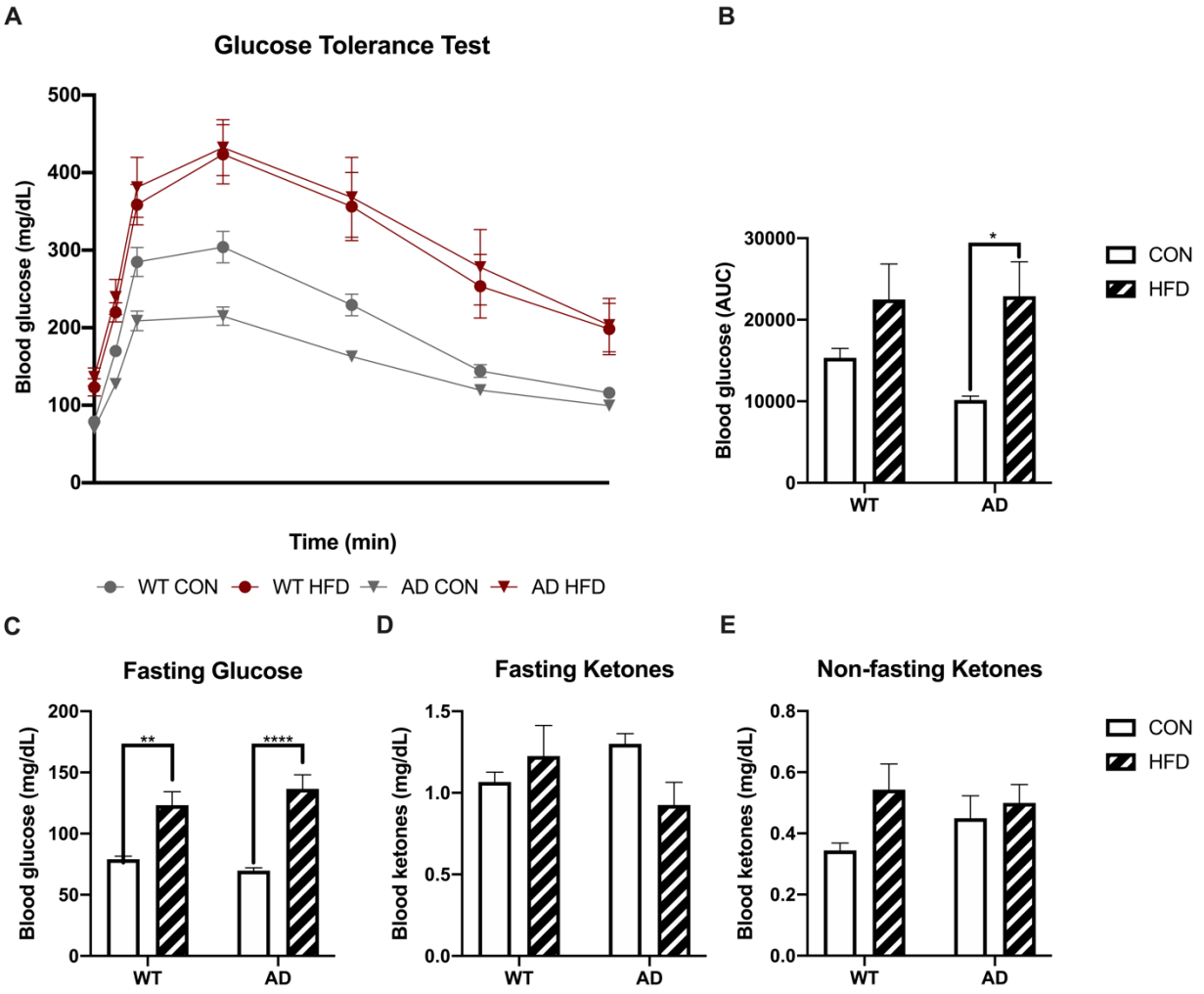


Figure 20. HFD induced hyperglycemia and affected glucose metabolism in 11-month-old mice.

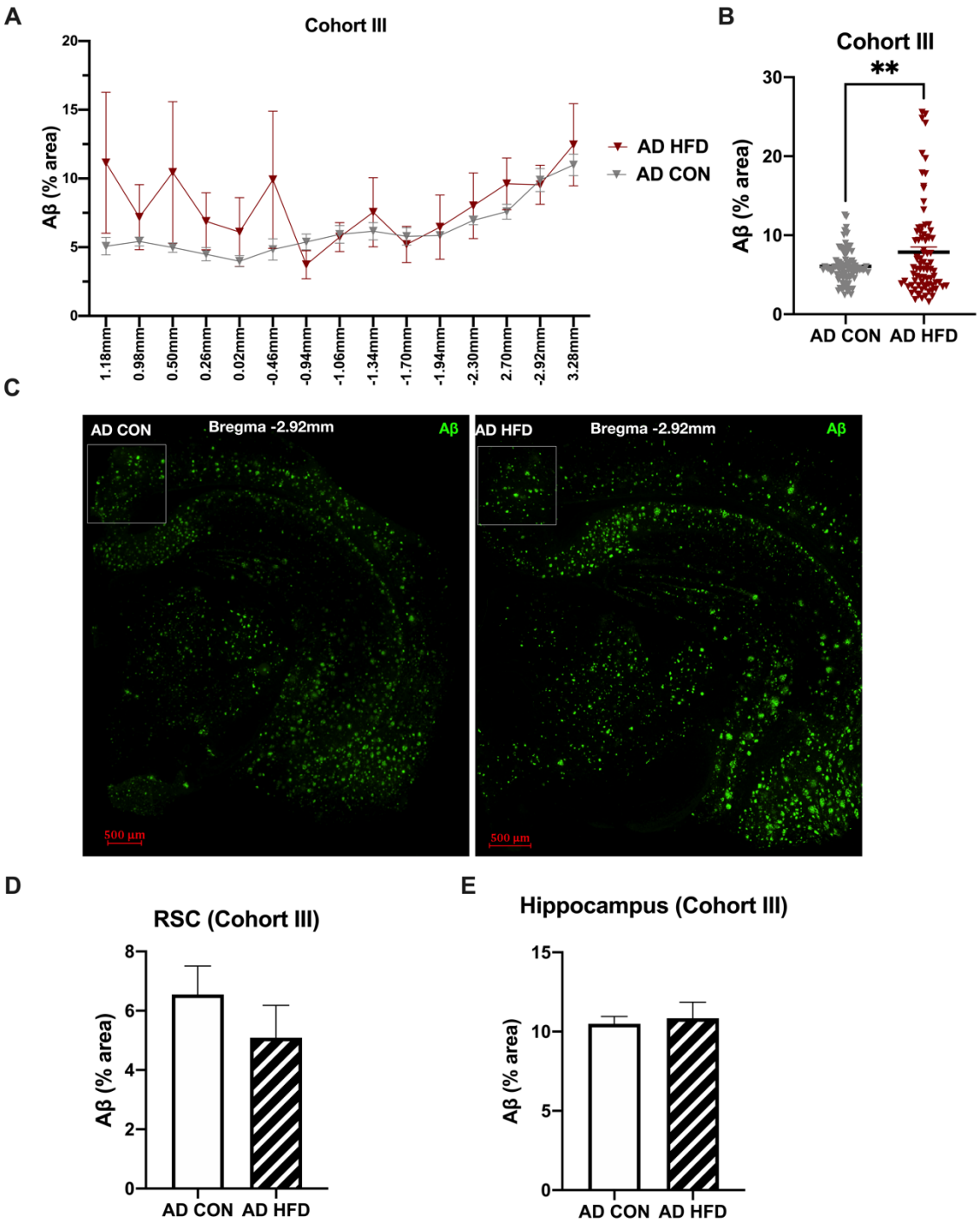
A HFD impaired glucose clearance in WT and AD mice after a glucose tolerance test (GTT). **B** HFD-fed AD, but not WT mice showed higher blood glucose levels after GTT, compared to CON-fed mice, as indicated by area under the curve (AUC). **C** HFD increased fasting blood glucose levels in WT and AD mice. HFD did not affect fasting (**D**) or non-fasting (**E**) blood ketone levels in WT and AD mice. n=8-9 mice per group. Results are from one representative experiment. Statistical analysis performed by two-way ANOVA. *p<0.05. **p<0.01.

HFD consumption starting at 6 months did not affect Aβ pathology or cognitive function in 11-month-old AD mice.

In Cohort III, where HFD consumption was delayed until 6 months-of-age, we saw an overall increase in Aβ brain staining in AD HFD mice compared to that of AD CON (Fig. 21A, B). When specifically comparing Aβ staining in the RSC of AD mice, there was no longer a protective effect of the HFD (Fig. 21C, D). There was no effect of HFD feeding on Aβ deposition in the hippocampus (Fig. 21C, E). These results indicate that delaying HFD feeding until after moderate AD-related pathology was developed failed to slow AD progression. HFD had no effect on locomotion during NOR training (Fig. 22A, B) or testing (Fig. 22C, D) in 11-month-old mice. We did not see any group differences in object exploration on the training day of NOR (Fig. 22E). HFD did not affect cognitive function by NOR in 11-month-old AD mice (Fig. 22F). Therefore, our findings suggest that delaying the onset of HFD feeding until 6 months, when AD pathology is widespread, does not alleviate AD-related pathology or cognitive dysfunction in mice.

Figure 21. HFD consumption starting at 6 months did not affect Aβ pathology in the RSC of AD mice.

A HFD consumption starting at 6 months until 11 months (Cohort III) increased Aβ deposition throughout the brains of 11-month-old AD mice. **B** Quantification of Aβ staining throughout the brains of Cohort III 11-month-old AD mice showed an increase in overall Aβ pathology. Each symbol represents a single Bregma point. **C** Representative images of Aβ staining in 11-month-old mice. Staining was quantified in the RSC and hippocampus. WT mice had no detectable Aβ deposits (not shown). Scale bar=500 μm. **D, E** In Cohort III, delaying HFD feeding until 6 months-of-age did not significantly decrease Aβ plaque deposition in the RSC or hippocampus. For **A** and **B**, n=4-9 mice per group. For **E-G**, n=8 mice per group. Results are from one representative experiment. Statistical analysis performed by Student's t-test. **p<0.01.


A
Cohort III
Aβ (% area)
20
15
10
5
0
1.18mm
0.98mm
0.50mm
0.26mm
0.02mm
-0.46mm
-0.94mm
-1.06mm
-1.34mm
-1.70mm
-1.94mm
-2.30mm
2.70mm
-2.92mm
3.28mm
AD HFD
AD CON
B
Cohort III
**
Aβ (% area)
30
20
10
0
AD CON
AD HFD
C
AD CON
Bregma -2.92mm
Aβ
500 µm
AD HFD
Bregma -2.92mm
Aβ
500 µm
D
RSC (Cohort III)
Aβ (% area)
8
6
4
2
0
AD CON
AD HFD
E
Hippocampus (Cohort III)
Aβ (% area)
15
10
5
0
AD CON
AD HFD

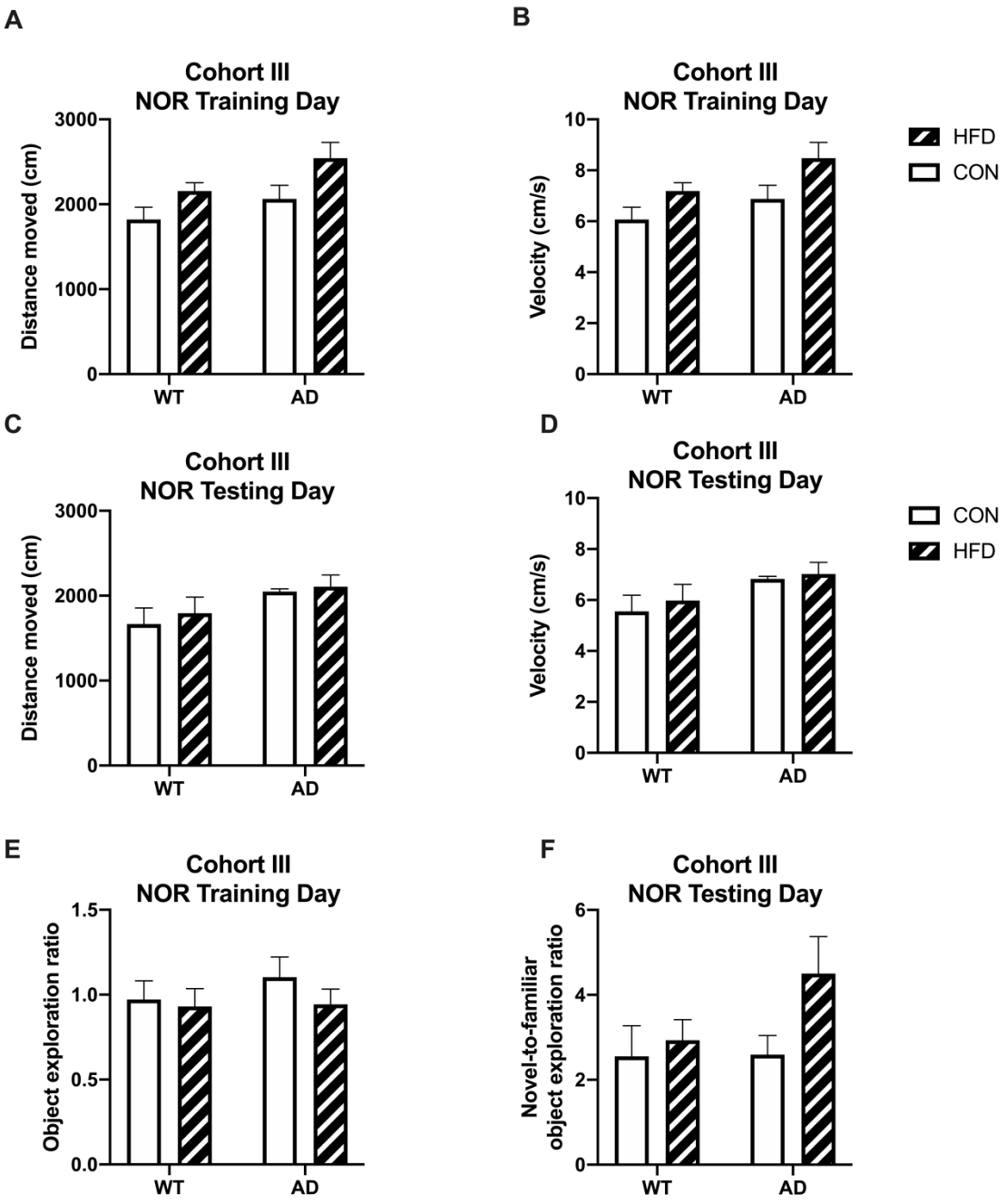


Figure 22. HFD consumption starting at 6 months did not affect cognition in 11-month-old AD mice.

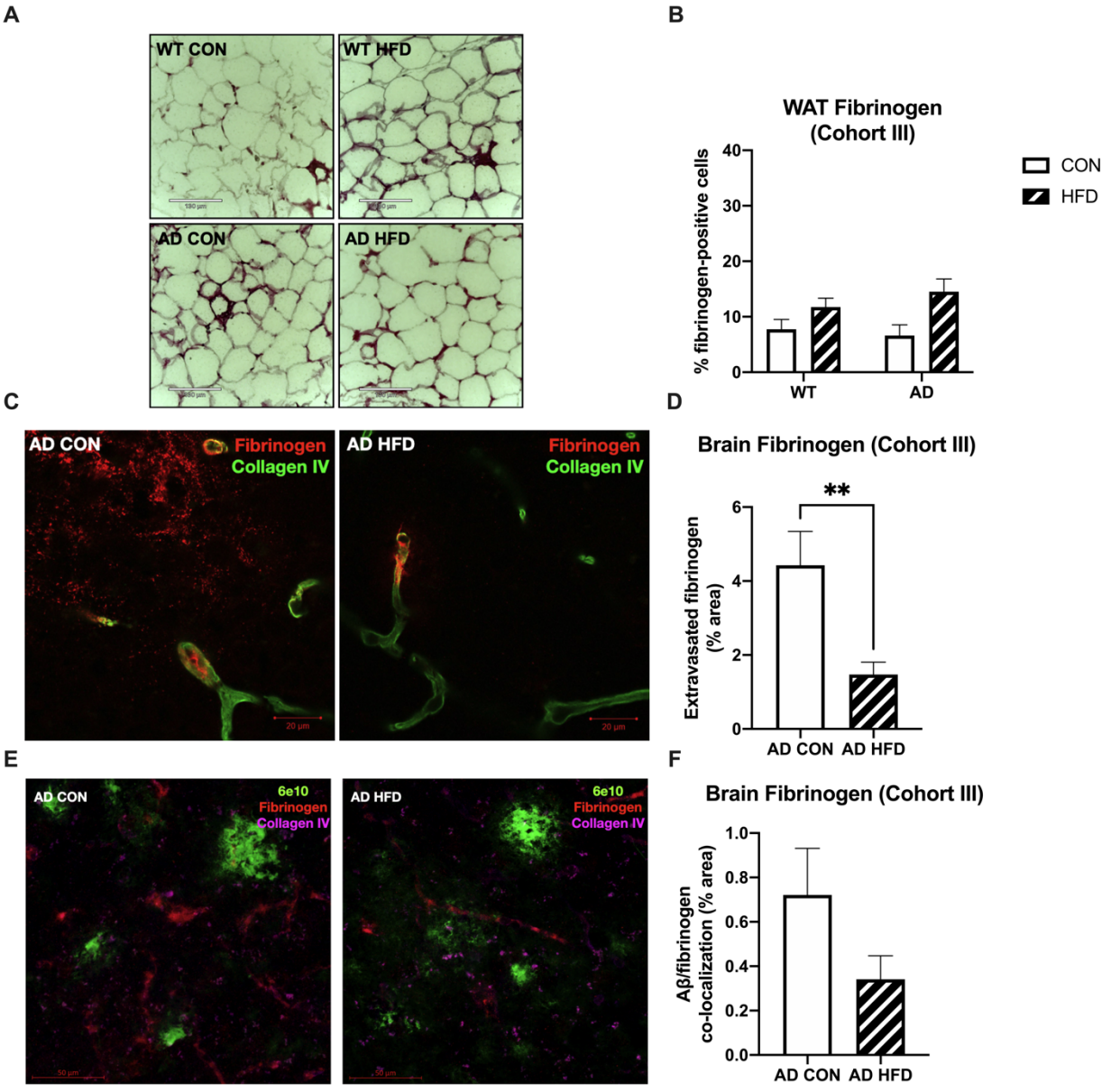
HFD did not affect the total distance moved or velocity during novel object recognition (NOR) training (**A, B**). HFD reduced the total distance moved and velocity in AD mice during NOR testing (**C, D**). **E** No differences in the object exploration ratio were present between groups on the training day of NOR test, indicating no place preference. **F** HFD did not affect object exploration in AD mice on the testing day of the NOR test. Results are from one representative experiment. n=8-9 mice per group. Statistical analysis performed by two-way ANOVA.

Delayed onset of HFD consumption until 6 months-of-age reduced fibrinogen extravasation into the brains of AD mice.

In Cohort III, where the onset of HFD was delayed until 6 months, HFD did not significantly affect the percentage of fibrinogen-positive cells in the WAT of 11-month-old WT and AD mice (Fig. 23A, B). Additionally, we found evidence of fibrinogen deposition in the WAT of CON-fed WT and AD mice (Fig. 23A), indicating that fibrinogen deposition in peripheral tissues might accompany aging in the absence of metabolic insults. Interestingly, HFD still significantly reduced fibrinogen extravasation into the brain parenchyma of 11-month-old AD mice (Fig. 23C, D), indicating that delayed-onset HFD improved BBB integrity even after AD-like pathology had begun but was unable to reverse or slow Aβ deposition. HFD did not affect Aβ/fibrinogen co-deposition in the RSC of 11-month-old AD mice (Fig. 23E, F) although a small non-significant decrease in Aβ/fibrinogen co-localization was observed in AD HFD mice. Overall, these results suggest that HFD improved BBB integrity and reduced fibrinogen extravasation into the brain parenchyma despite increasing Aβ deposition throughout the brain, which might indicate that Aβ deposition and fibrinogen deposition represent two distinct pathological processes in the model that we are using, although Aβ/fibrinogen co-deposits have been shown to exacerbate AD-related phenotypes.

Figure 23. HFD consumption starting at 6 months reduced fibrinogen extravasation into the RSC of 11-month-old mice.

A Representative images of WAT fibrinogen staining in 11-month-old mice after 20 weeks of experimental diet. **B** HFD did not affect the percentage of fibrinogen-positive cells in WAT of 11-month-old WT and AD mice. **C** Representative images of fibrinogen staining (red) show fibrinogen extravasation from collagen IV-positive blood vessels (green) into the RSC of 11-month-old AD CON mice, but not AD HFD mice. **D** HFD significantly reduced fibrinogen staining outside of blood vessels in RSC of 11-month-old AD mice. **E** Representative triple-stained images of Aβ deposits (green, 6E10) and fibrinogen extravasation (red) from collagen IV-positive blood vessels (purple) in the brain parenchyma of 11-month-old AD CON, but not AD HFD mice. **F** HFD had a small but insignificant effect on the interaction between Aβ and fibrinogen in the RSC of 11-month-old AD mice. For **A** and **B**, n=4-5 mice per group, 3 slices per animal. For **C** and **D**, n=8 mice per group, 3 slices per animal. For **E** and **F**, n=5-6 mice per group, 3 slices per animal. Results are from one representative experiment. Statistical analysis performed by two-way ANOVA. **p<0.01. Scale bar=130μm in **A**; 20 μm in **C;** 50 μm in **E**.


A
WT CON
WT HFD
AD CON
AD HFD
B
WAT Fibrinogen
(Cohort III)
% fibrinogen-positive cells
40
30
20
10
0
WT
AD
CON
HFD
C
AD CON
Fibrinogen
Collagen IV
AD HFD
Fibrinogen
Collagen IV
20 μm
D
Brain Fibrinogen (Cohort III)
**
Extravasated fibrinogen
(% area)
6
4
2
0
AD CON
AD HFD
E
AD CON
6e10
Fibrinogen
Collagen IV
AD HFD
6e10
Fibrinogen
Collagen IV
50 μm
F
Brain Fibrinogen (Cohort III)
Aβ/fibrinogen
co-localization (% area)
1.0
0.8
0.6
0.4
0.2
0.0
AD CON
AD HFD

Chapter 6: Discussion and Conclusions

The goal of our study was to determine the effect of varying the timeline of HFD consumption on AD-related pathology and cognitive function in Tg6799 AD mice. In this rapid progression animal model of AD, extracellular plaque deposition begins at 2 months and is readily detectable throughout the cortex and hippocampus by 6 months [30]. Furthermore, Tg6799 mice display evidence of spatial memory deficits as early as 4 months-of-age [30].

We initially administered HFD to Tg6799 AD mice before the onset of extracellular Aβ deposition or cognitive deficits. Contrary to some previous reports, HFD consumption significantly reduced the percent area covered by Aβ plaques in the RSC and hippocampus as well as throughout the whole brain of 6-month-old AD mice when compared to their CON-fed AD littermates. Importantly, HFD improved object recognition memory, which indicates an improvement in cognitive function. Surprisingly, when we delayed the onset of HFD until 3 months-of-age, after Aβ starts accumulating, HFD similarly reduced Aβ plaque deposition in the RSC and improved cognitive function in 8-month-old AD mice. However, HFD did not improve Aβ pathology or cognitive function in 11-month-old AD mice when HFD consumption started at 6 months-of-age. In fact, Aβ pathology was exacerbated across the entire brain when HFD feeding occurred after AD pathology had already begun. Thus, the timing of HFD consumption is critical for improving AD-related pathology and cognition, as HFD does little to rescue AD-related phenotypes after Aβ pathology has already spread throughout the brain.

In this study, consumption of HFD at an early age led to region-specific as well as widespread reduction of Aβ deposition throughout the brains of AD mice. One of the specific brain areas affected by dietary fat consumption was the RSC, which has important reciprocal connections with several brains regions, including hippocampus [85], visuospatial cortex [86], prefrontal cortex [87], and posterior secondary motor cortex [88]. The connectivity pattern of the RSC is consistent with its role in cognitive function [89-92]. The RSC is thought to play a critical role in AD pathophysiology as it is one of the first regions to undergo pathological changes in patients [93, 94]. Pathological changes in the RSC are also present in mouse models of AD. Tg2576 AD mice show aberrant changes in the markers of cellular activity in RSC before the formation of overt Aβ pathology [95]. In Tg6799 AD mice, increased accumulation of Aβ in the RSC is accompanied by an impairment in object recognition memory as early as at 4 months-of-age [96]. Taken together, these results indicate that pathological changes in the RSC may reflect some of the earliest AD-related processes and impact cognitive function in mouse models of AD. In our study, HFD consumption reduced Aβ deposition in the RSC and improved object recognition memory in 6- and 8-month-old AD mice. However, delaying the onset of HFD consumption until after mice display overt Aβ accumulation and cognitive deficits did not improve object recognition memory. HFD consumption did not affect WT mouse learning and memory behaviors in novel object recognition tests nor were there any changes observed in brain pathology of WT mice. Therefore, our results indicate that early consumption of HFD led to a reduction in Aβ accumulation in the RSC and hippocampus which contributed to an improvement in cognitive performance in AD mice.

In addition to hallmark AD pathologies, AD is also associated with vascular abnormalities [97, 98], such as parenchymal deposition of fibrinogen, a critical component of the blood coagulation

cascade [75, 99-101]. Fibrinogen is a glycoprotein found in large quantities in blood [102]. Fibrinogen is normally excluded from the brain by the BBB. However, AD patients and mouse models show a loss of BBB integrity [103-107], which promotes fibrinogen extravasation into the brain. We and others have previously shown that fibrinogen extravasation correlates with the degree of Aβ pathology in AD mice and patients [99, 108-110]. Due to its potent pro-inflammatory properties and direct interaction with Aβ [80, 111-113], extravascular fibrinogen may contribute to and/or promote neuroinflammatory responses and neuronal dysfunction [100, 114]. In fact, fibrin deposition is present in areas of the brain with prominent synaptic degeneration [99]. In this study, we found extravasated fibrinogen in the RSC of 6-month-old AD mice (Cohort I), while their WT littermates showed none. However, chronic HFD consumption reduced fibrinogen extravasation in AD mice. Surprisingly, 20-week HFD consumption also reduced fibrinogen extravasation in the RSC of 8- and 11-month-old AD mice (Cohorts II and III), but HFD only reduced Aβ deposition in the RSC of 8-, but not 11-, month mice. This result indicates that a reduction in extravascular fibrinogen does not directly reflect a reduction in Aβ. In addition, we found minimal Aβ/fibrinogen co-deposits in the RSC of AD HFD mice at 6-, 8-, and 11-months-of-age. This result also suggests that Aβ accumulation occurs prior to BBB disruption and fibrinogen extravasation into the brain parenchyma. Therefore, the Aβ/fibrinogen interaction resulting in their co-deposition in the brain might represent a later stage in the development of AD-related pathology. Additionally, HFD failed to induce an improvement in object recognition memory in 11-month-old AD mice. This result suggests that chronic HFD consumption is not sufficient to preserve cognitive function in older AD mice if HFD feeding starts after significant Aβ deposition, despite potential neuroprotective effects that accompany reduced BBB permeability to fibrinogen [99]. Future studies will determine whether HFD-induced reduction in fibrinogen extravasation protects the brains of AD mice from synaptic degeneration with HFD feeding starting at 1, 3, or 6 months-of-age.

One of the major roles of fibrinogen in the circulatory systems is to promote blood clotting. In response to injury, fibrinogen is converted to fibrin, the major component of blood clots [115]. This response can be achieved via the activation of the plasma contact system. The activation of the plasma contact system is also associated with AD pathogenesis. In AD, Aβ42 can bind to and activate FXII [83]. Activated FXII cleaves factor XI (FXI), which, in turn, sets off a series of activation events that eventually result in fibrin clot formation [116]. In addition, Aβ has been shown to promote thrombin generation, which is required for fibrin clot formation [83]. Since our data demonstrated that HFD reduces the extravasation of fibrinogen from blood vessels into the brain parenchyma, we tested the hypothesis that HFD decreases plasma contact system activation in 6-month-old WT and AD mice. However, the results of this study demonstrate that HFD does not affect plasma contact system activation in either WT or AD mice, since we did not detect any differences in plasma levels of HK, PK, or FXII in 6-month-old mice between HFD-fed and CON-fed mice. Additionally, HFD did not affect the activity of plasma kallikrein. Hence, our data indicate that the reduction in extravascular fibrinogen in the cortex of 6-month-old AD mice is due to improved BBB integrity and not a reduction in the activation of the plasma contact system.

Finally, we determined that HFD induces transcriptome changes in the cortex of HFD-fed AD mice, when compared to AD CON mice. HFD affected the expression of only 7 genes in WT mice, when compared to WT CON mice. However, in AD mice HFD upregulated a total of 30 genes and

downregulated 13 genes, when compared to AD CON mice. Additional pairwise comparisons revealed that AD genotype affected the expression of 226 genes in CON-fed mice. However, in HFD-fed AD mice, the total number of differentially expressed genes was increased to 354. These results indicate that transcriptome changes in HFD-fed AD mice were driven by an interaction between their diet and genotype. HFD-induced transcriptome changes in AD mice were distinct from those induced by HFD in WT mice. Gene ontology analysis of differentially expressed genes in HFD-fed AD mice showed that the majority of upregulated transcripts are involved in protein folding. Additional gene ontology terms included cell redox homeostasis, response to nutrient, and response to endoplasmic reticulum stress.

HFD consumption also significantly reduced the expression of human *APP* in the cortex of AD mice but had no effect on the expression of human *PSEN1*. APP, the parent molecule of the Aβ peptide, can be processed in different ways to produce various species[117]. The non-amyloidogenic cleavage of APP, initiated by α-secretase, generates a large soluble APP-α fragment (sAPPα), while the amyloidogenic cleavage of APP, initiated by β-secretase, generates a soluble APP-β fragment (sAPPβ) and an N-terminally intact Aβ peptide. In this study, we report reduced deposition of cortical Aβ in 6-month-old AD HFD mice which was concomitant with reduced expression of human *APP* in the cortex of these mice. This indicates that long-term HFD consumption might affect APP transcription resulting in reduced Aβ accumulation. However, we cannot exclude the possibility that APP processing or microglial clearance of Aβ is also affected by HFD. Additionally, HFD affected the expression of several genes associated with protein folding, such as *Cryab*, a small heat-shock protein that exhibits molecular chaperone activity, thus raising the possibility that such chaperone activity might also prevent Aβ aggregation and further reduce Aβ in the cortex of AD mice [118].

Our results are consistent with previous studies that have reported HFD-induced improvement in BBB function in AD mice. For example, in rodent MRI studies HFD significantly reduces BBB permeability to the contrast agent and decreases the volume of lateral ventricles in Tg2576 AD mice, indicating a reduction in brain atrophy [24]. This reduction in BBB leakage is accompanied by a significant improvement in learning in HFD-fed AD mice. However, the protective effect of HFD on BBB function might depend on HFD composition. For example, Theriault et al. reported that a sucrose-rich "Western diet" containing 42% kcal from fat and 42.7% kcal from carbohydrates does not affect BBB permeability and exacerbates AD-related cognitive decline in APPswe/PS1 mice [119]. In addition, another Western diet high in saturated fat and sucrose increases Aβ plaque load and microglial density in APP/PS1 mice without affecting short-term memory [29]. These results indicate that a high-fat, low-carbohydrate diet – like the one used in our study – may be protective against AD-induced BBB damage, while high-fat diets containing high levels of carbohydrates and/or sucrose have deleterious effects on AD pathophysiology.

Conclusions

Calorically dense diets, specifically those high in fat, provide an opportunity to model diet-induced obesity and investigate the effect of dietary fats on AD-related pathology and cognitive dysfunction in AD mouse lines. However, due to the 1) diversity of AD mouse models, 2) wide range of commercially available high fat diets, 3) feeding protocols that vary the timelines of HFD consumption, and 4) sex differences in both diet-induced obesity and AD pathophysiology,

published studies have failed to yield conclusive results. While many studies report HFD-induced worsening of AD-related pathology, earlier onset and more severe cognitive decline, others report no association between HFD and AD. Moreover, this study as well as others show that HFD can actually exert a beneficial effect on AD-related pathologies. Therefore, the translational potential of such studies remains to be determined. In this study we show that chronic HFD consumption reduced AD-related pathology, including Aβ accumulation, fibrinogen extravasation, and cognitive dysfunction in 6- and 8-month-old AD mice when HFD intake started at or before 3 months-of-age (before or during early-stage disease; Fig. 24A). In 6-month-old mice, decreased Aβ accumulation might be explained by the decreased transcription of APP, the parent molecule of Aβ, in HFD-fed AD mice (Fig. 24B). Additionally, HFD decreased fibrinogen extravasation into the brain parenchyma in both 6- and 8-month old mice, indicating that HFD consumed before or during early-stage disease reduced BBB permeability in AD mice (Fig. 24B). HFD-fed 6- and 8-month-old AD mice also showed improved object recognition memory in the NOR test, suggesting that improved cognition in these younger animals might be due to the decrease in synaptic damage induced by the alleviated Aβ load and reduced fibrinogen extravasation into the brain parenchyma. Delaying the onset of HFD until 6 months (moderate disease pathology) voided the protective effect of HFD, since HFD did not reduce Aβ plaque deposition or rescue cognitive decline in 11-month-old AD mice (Fig. 24A). However, the delayed onset of HFD feeding still reduced the extravasation of fibrinogen into the brain parenchyma, thus raising the possibility that a diet high in fat helped maintain or repair BBB integrity even after AD mice developed overt Aβ pathology and cognitive deficits.

What remains to be studied is the effect of consuming specific dietary fats on AD pathogenesis in mouse models. Regardless, the heterogeneity of the findings summarized in this work and as well as the juxtaposition between the results reported here and elsewhere is in line with the clinical heterogeneity present in the AD patient population. In fact, clinicians often advocate for individualized treatment plans for AD patients, including customized nutritional interventions, that reflect the complexity of AD pathophysiology, which is often affected by modifiable risk factors [120]. Thus, to achieve AD risk reduction and prevention as well as early intervention, clinical decisions about nutritional interventions should be made based on patients' individual clinical profiles and include thorough evaluations and nutritional counseling.

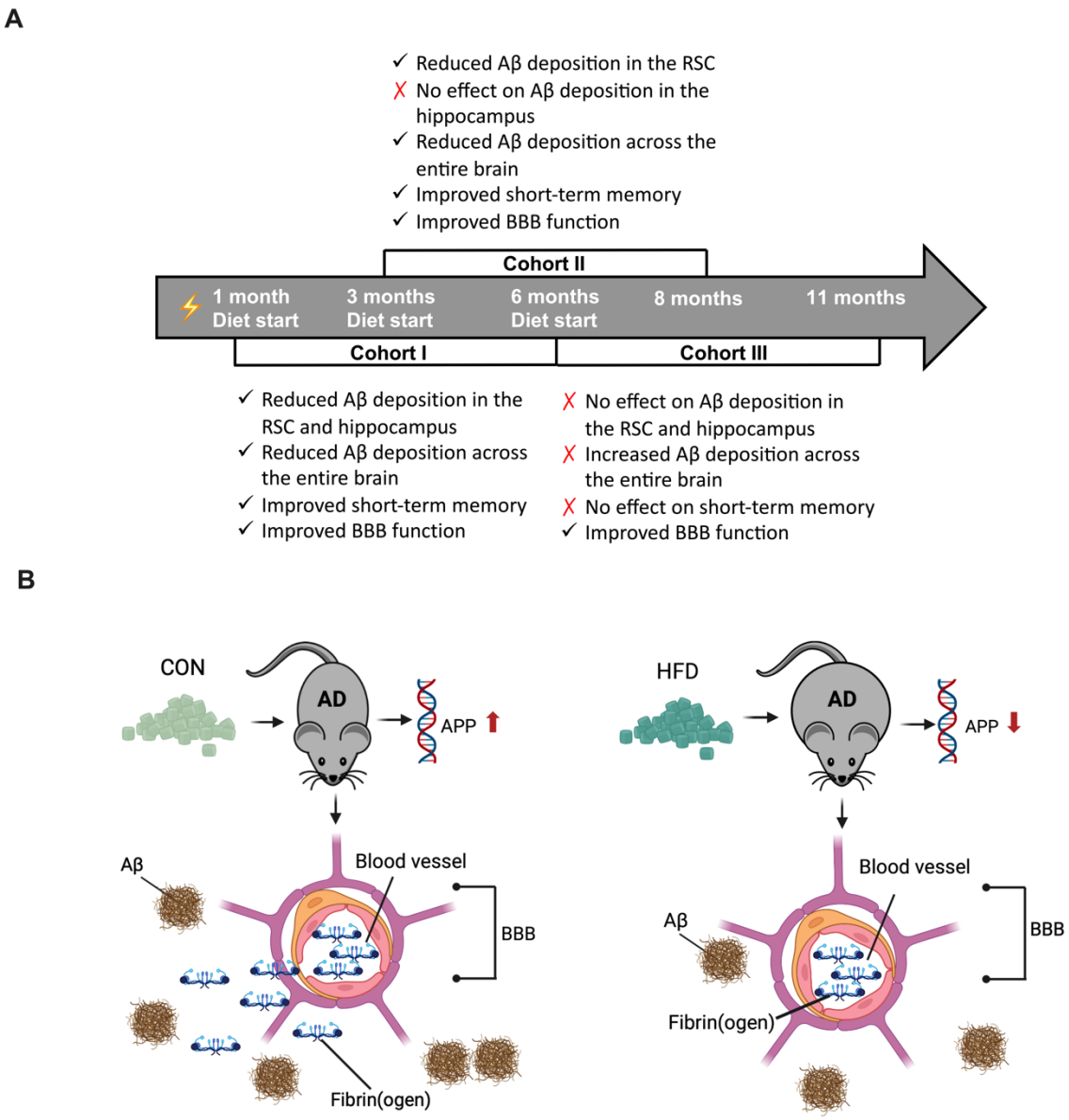


Figure 24. Early dietary fat supplementation ameliorated AD-related pathology and improved cognitive function in mice.
A Schematic shows feeding timeline in WT and AD mice in Cohorts I, II, and III. **B** Schematic shows the effect of CON and HFD on APP transcription and BBB permeability. The components of the blood brain barrier (BBB) are depicted in purple (astrocytic end-feet), yellow (pericytes), and pink (endothelium).

References

1. Harper, S., C.A. Riddell, and N.B. King, *Declining Life Expectancy in the United States: Missing the Trees for the Forest.* Annu Rev Public Health, 2021. 42: p. 381-403.
2. Rajan, K.B., et al., *Population estimate of people with clinical Alzheimer's disease and mild cognitive impairment in the United States (2020-2060).* Alzheimers Dement, 2021. 17(12): p. 1966-1975.
3. *2021 Alzheimer's disease facts and figures.* Alzheimers Dement, 2021. 17(3): p. 327-406.
4. Ahmad, F.B., et al., *Provisional Mortality Data - United States, 2020.* MMWR Morb Mortal Wkly Rep, 2021. 70(14): p. 519-522.
5. Herrmann, N., et al., *Current and emerging drug treatment options for Alzheimer's disease: a systematic review.* Drugs, 2011. 71(15): p. 2031-65.
6. Carreiras, M.C., et al., *The multifactorial nature of Alzheimer's disease for developing potential therapeutics.* Curr Top Med Chem, 2013. 13(15): p. 1745-70.
7. Bekris, L.M., et al., *Genetics of Alzheimer disease.* J Geriatr Psychiatry Neurol, 2010. 23(4): p. 213-27.
8. Coon, K.D., et al., *A high-density whole-genome association study reveals that APOE is the major susceptibility gene for sporadic late-onset Alzheimer's disease.* J Clin Psychiatry, 2007. 68(4): p. 613-8.
9. Almeida, R.P., et al., *Effect of Cognitive Reserve on Age-Related Changes in Cerebrospinal Fluid Biomarkers of Alzheimer Disease.* JAMA Neurol, 2015. 72(6): p. 699-706.
10. Sommerlad, A., et al., *Association of social contact with dementia and cognition: 28-year follow-up of the Whitehall II cohort study.* PLoS Med, 2019. 16(8): p. e1002862.
11. Gardner, R.C., et al., *Dementia risk after traumatic brain injury vs nonbrain trauma: the role of age and severity.* JAMA Neurol, 2014. 71(12): p. 1490-7.
12. Newman, A.B., et al., *Dementia and Alzheimer's disease incidence in relationship to cardiovascular disease in the Cardiovascular Health Study cohort.* J Am Geriatr Soc, 2005. 53(7): p. 1101-7.
13. Gustafson, D., et al., *An 18-year follow-up of overweight and risk of Alzheimer disease.* Arch Intern Med, 2003. 163(13): p. 1524-8.
14. Beydoun, M.A., et al., *Association of adiposity status and changes in early to mid-adulthood with incidence of Alzheimer's disease.* Am J Epidemiol, 2008. 168(10): p. 1179-89.
15. Xu, W.L., et al., *Midlife overweight and obesity increase late-life dementia risk: a population-based twin study.* Neurology, 2011. 76(18): p. 1568-74.
16. Loef, M. and H. Walach, *Midlife obesity and dementia: meta-analysis and adjusted forecast of dementia prevalence in the United States and China.* Obesity (Silver Spring), 2013. 21(1): p. E51-5.
17. Pedditzi, E., R. Peters, and N. Beckett, *The risk of overweight/obesity in mid-life and late life for the development of dementia: a systematic review and meta-analysis of longitudinal studies.* Age Ageing, 2016. 45(1): p. 14-21.
18. Qizilbash, N., et al., *BMI and risk of dementia in two million people over two decades: a retrospective cohort study.* Lancet Diabetes Endocrinol, 2015. 3(6): p. 431-436.
19. Kivimaki, M., et al., *Body mass index and risk of dementia: Analysis of individual-level data from 1.3 million individuals.* Alzheimers Dement, 2018. 14(5): p. 601-609.

20. Wakabayashi, T., et al., *Differential effects of diet- and genetically-induced brain insulin resistance on amyloid pathology in a mouse model of Alzheimer's disease.* Mol Neurodegener, 2019. 14(1): p. 15.
21. Knight, E.M., et al., *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, 2014. 35(8): p. 1821-32.
22. Ho, L., et al., *Diet-induced insulin resistance promotes amyloidosis in a transgenic mouse model of Alzheimer's disease.* Faseb j, 2004. 18(7): p. 902-4.
23. Herculano, B., et al., *beta-alanyl-L-histidine rescues cognitive deficits caused by feeding a high fat diet in a transgenic mouse model of Alzheimer's disease.* J Alzheimers Dis, 2013. 33(4): p. 983-97.
24. Elhaik Goldman, S., et al., *High-fat diet protects the blood-brain barrier in an Alzheimer's disease mouse model.* Aging Cell, 2018. 17(5): p. e12818.
25. Amelianchik, A., et al., *The protective effect of early dietary fat consumption on Alzheimer's disease-related pathology and cognitive function in mice.* Alzheimers Dement (N Y), 2021. 7(1): p. e12173.
26. Radde, R., et al., *Abeta42-driven cerebral amyloidosis in transgenic mice reveals early and robust pathology.* EMBO Rep, 2006. 7(9): p. 940-6.
27. Serneels, L., et al., *gamma-Secretase heterogeneity in the Aph1 subunit: relevance for Alzheimer's disease.* Science, 2009. 324(5927): p. 639-42.
28. Walker, J.M., et al., *Reversal of high fat diet-induced obesity improves glucose tolerance, inflammatory response, beta-amyloid accumulation and cognitive decline in the APP/PSEN1 mouse model of Alzheimer's disease.* Neurobiol Dis, 2017. 100: p. 87-98.
29. Bracko, O., et al., *High fat diet worsens Alzheimer's disease-related behavioral abnormalities and neuropathology in APP/PS1 mice, but not by synergistically decreasing cerebral blood flow.* Sci Rep, 2020. 10(1): p. 9884.
30. Oakley, H., et al., *Intraneuronal beta-amyloid aggregates, neurodegeneration, and neuron loss in transgenic mice with five familial Alzheimer's disease mutations: potential factors in amyloid plaque formation.* J Neurosci, 2006. 26(40): p. 10129-40.
31. Jawhar, S., et al., *Motor deficits, neuron loss, and reduced anxiety coinciding with axonal degeneration and intraneuronal Abeta aggregation in the 5XFAD mouse model of Alzheimer's disease.* Neurobiol Aging, 2012. 33(1): p. 196 e29-40.
32. Richard, B.C., et al., *Gene Dosage Dependent Aggravation of the Neurological Phenotype in the 5XFAD Mouse Model of Alzheimer's Disease.* J Alzheimers Dis, 2015. 45(4): p. 1223-36.
33. Medrano-Jimenez, E., et al., *Malva parviflora extract ameliorates the deleterious effects of a high fat diet on the cognitive deficit in a mouse model of Alzheimer's disease by restoring microglial function via a PPAR-gamma-dependent mechanism.* J Neuroinflammation, 2019. 16(1): p. 143.
34. Reilly, A.M., et al., *Metabolic Defects Caused by High-Fat Diet Modify Disease Risk through Inflammatory and Amyloidogenic Pathways in a Mouse Model of Alzheimer's Disease.* Nutrients, 2020. 12(10).
35. Lin, B., et al., *High-Fat-Diet Intake Enhances Cerebral Amyloid Angiopathy and Cognitive Impairment in a Mouse Model of Alzheimer's Disease, Independently of Metabolic Disorders.* J Am Heart Assoc, 2016. 5(6).

36. Sturchler-Pierrat, C., et al., *Two amyloid precursor protein transgenic mouse models with Alzheimer disease-like pathology.* Proc Natl Acad Sci U S A, 1997. 94(24): p. 13287-92.
37. Van Dam, D., et al., *Age-dependent cognitive decline in the APP23 model precedes amyloid deposition.* Eur J Neurosci, 2003. 17(2): p. 388-96.
38. Nam, K.N., et al., *Effect of high fat diet on phenotype, brain transcriptome and lipidome in Alzheimer's model mice.* Sci Rep, 2017. 7(1): p. 4307.
39. Saito, T., et al., *Single App knock-in mouse models of Alzheimer's disease.* Nat Neurosci, 2014. 17(5): p. 661-3.
40. Masuda, A., et al., *Cognitive deficits in single App knock-in mouse models.* Neurobiol Learn Mem, 2016. 135: p. 73-82.
41. Salas, I.H., et al., *High fat diet treatment impairs hippocampal long-term potentiation without alterations of the core neuropathological features of Alzheimer disease.* Neurobiol Dis, 2018. 113: p. 82-96.
42. Macklin, L., et al., *Glucose tolerance and insulin sensitivity are impaired in APP/PS1 transgenic mice prior to amyloid plaque pathogenesis and cognitive decline.* Exp Gerontol, 2017. 88: p. 9-18.
43. Mazzei, G., et al., *A high-fat diet exacerbates the Alzheimer's disease pathology in the hippocampus of the App(NL-F/NL-F) knock-in mouse model.* Aging Cell, 2021. 20(8): p. e13429.
44. Oddo, S., et al., *Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular Abeta and synaptic dysfunction.* Neuron, 2003. 39(3): p. 409-21.
45. Billings, L.M., et al., *Intraneuronal Abeta causes the onset of early Alzheimer's disease-related cognitive deficits in transgenic mice.* Neuron, 2005. 45(5): p. 675-88.
46. Rollins, C.P.E., et al., *Contributions of a high-fat diet to Alzheimer's disease-related decline: A longitudinal behavioural and structural neuroimaging study in mouse models.* Neuroimage Clin, 2019. 21: p. 101606.
47. Farrer, L.A., et al., *Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. APOE and Alzheimer Disease Meta Analysis Consortium.* JAMA, 1997. 278(16): p. 1349-56.
48. Sullivan, P.M., et al., *Targeted replacement of the mouse apolipoprotein E gene with the common human APOE3 allele enhances diet-induced hypercholesterolemia and atherosclerosis.* J Biol Chem, 1997. 272(29): p. 17972-80.
49. Lewandowski, C.T., J. Maldonado Weng, and M.J. LaDu, *Alzheimer's disease pathology in APOE transgenic mouse models: The Who, What, When, Where, Why, and How.* Neurobiol Dis, 2020. 139: p. 104811.
50. Jones, N.S., K.Q. Watson, and G.W. Rebeck, *Metabolic Disturbances of a High-Fat Diet Are Dependent on APOE Genotype and Sex.* eNeuro, 2019. 6(5).
51. Janssen, C.I., et al., *The Effect of a High-Fat Diet on Brain Plasticity, Inflammation and Cognition in Female ApoE4-Knockin and ApoE-Knockout Mice.* PLoS One, 2016. 11(5): p. e0155307.
52. Nam, K.N., et al., *Integrated approach reveals diet, APOE genotype and sex affect immune response in APP mice.* Biochim Biophys Acta Mol Basis Dis, 2018. 1864(1): p. 152-161.
53. Martin, S.A., et al., *Maternal high-fat diet worsens memory deficits in the triple-transgenic (3xTgAD) mouse model of Alzheimer's disease.* PLoS One, 2014. 9(6): p. e99226.
54. Tozuka, Y., et al., *Maternal obesity impairs hippocampal BDNF production and spatial learning performance in young mouse offspring.* Neurochem Int, 2010. 57(3): p. 235-47.

55. Hatanaka, Y., K. Wada, and T. Kabuta, *Maternal high-fat diet leads to persistent synaptic instability in mouse offspring via oxidative stress during lactation.* Neurochem Int, 2016. 97: p. 99-108.
56. Yu, H., et al., *Long-term effects of high lipid and high energy diet on serum lipid, brain fatty acid composition, and memory and learning ability in mice.* Int J Dev Neurosci, 2010. 28(3): p. 271-6.
57. Hsiao, K., et al., *Correlative memory deficits, Abeta elevation, and amyloid plaques in transgenic mice.* Science, 1996. 274(5284): p. 99-102.
58. Kawarabayashi, T., et al., *Age-dependent changes in brain, CSF, and plasma amyloid (beta) protein in the Tg2576 transgenic mouse model of Alzheimer's disease.* J Neurosci, 2001. 21(2): p. 372-81.
59. Belfiore, R., et al., *Temporal and regional progression of Alzheimer's disease-like pathology in 3xTg-AD mice.* Aging Cell, 2019. 18(1): p. e12873.
60. Ishii, M., et al., *Transgenic mice overexpressing amyloid precursor protein exhibit early metabolic deficits and a pathologically low leptin state associated with hypothalamic dysfunction in arcuate neuropeptide Y neurons.* J Neurosci, 2014. 34(27): p. 9096-106.
61. Di Meco, A. and D. Pratico, *Early-life exposure to high-fat diet influences brain health in aging mice.* Aging Cell, 2019. 18(6): p. e13040.
62. Hummel, K.P., M.M. Dickie, and D.L. Coleman, *Diabetes, a new mutation in the mouse.* Science, 1966. 153(3740): p. 1127-8.
63. Hierro-Bujalance, C., et al., *Cell proliferation and neurogenesis alterations in Alzheimer's disease and diabetes mellitus mixed murine models.* J Neurochem, 2020. 154(6): p. 673-692.
64. Takeda, S., et al., *Diabetes-accelerated memory dysfunction via cerebrovascular inflammation and Abeta deposition in an Alzheimer mouse model with diabetes.* Proc Natl Acad Sci U S A, 2010. 107(15): p. 7036-41.
65. Ingalls, A.M., M.M. Dickie, and G.D. Snell, *Obese, a new mutation in the house mouse.* J Hered, 1950. 41(12): p. 317-8.
66. Coleman, D.L., *Effects of parabiosis of obese with diabetes and normal mice.* Diabetologia, 1973. 9(4): p. 294-8.
67. Zhang, Y., et al., *Positional cloning of the mouse obese gene and its human homologue.* Nature, 1994. 372(6505): p. 425-32.
68. Yamada, K., et al., *Abeta immunotherapy: intracerebral sequestration of Abeta by an anti-Abeta monoclonal antibody 266 with high affinity to soluble Abeta.* J Neurosci, 2009. 29(36): p. 11393-8.
69. Sah, S.K., et al., *Effect of high-fat diet on cognitive impairment in triple-transgenic mice model of Alzheimer's disease.* Biochem Biophys Res Commun, 2017. 493(1): p. 731-736.
70. Robison, L.S., et al., *Role of sex and high-fat diet in metabolic and hypothalamic disturbances in the 3xTg-AD mouse model of Alzheimer's disease.* J Neuroinflammation, 2020. 17(1): p. 285.
71. Martins, I.V., et al., *Mitochondrial Abnormalities and Synaptic Loss Underlie Memory Deficits Seen in Mouse Models of Obesity and Alzheimer's Disease.* J Alzheimers Dis, 2017. 55(3): p. 915-932.
72. Pellizzon, M.A. and M.R. Ricci, *The common use of improper control diets in diet-induced metabolic disease research confounds data interpretation: the fiber factor.* Nutr Metab (Lond), 2018. 15: p. 3.

73. Mesnage, R., et al., *Laboratory Rodent Diets Contain Toxic Levels of Environmental Contaminants: Implications for Regulatory Tests.* PLoS One, 2015. 10(7): p. e0128429.
74. Holmdahl, R. and B. Malissen, *The need for littermate controls.* Eur J Immunol, 2012. 42(1): p. 45-7.
75. Cortes-Canteli, M., et al., *Fibrinogen and beta-amyloid association alters thrombosis and fibrinolysis: a possible contributing factor to Alzheimer's disease.* Neuron, 2010. 66(5): p. 695-709.
76. Kopec, A.M., et al., *Optimized solubilization of TRIzol-precipitated protein permits Western blotting analysis to maximize data available from brain tissue.* J Neurosci Methods, 2017. 280: p. 64-76.
77. Zamolodchikov, D., et al., *Activation of the factor XII-driven contact system in Alzheimer's disease patient and mouse model plasma.* Proc Natl Acad Sci U S A, 2015. 112(13): p. 4068-73.
78. Antunes, M. and G. Biala, *The novel object recognition memory: neurobiology, test procedure, and its modifications.* Cogn Process, 2012. 13(2): p. 93-110.
79. Kopec, A.K., et al., *Thrombin promotes diet-induced obesity through fibrin-driven inflammation.* J Clin Invest, 2017. 127(8): p. 3152-3166.
80. Ahn, H.J., et al., *Alzheimer's disease peptide beta-amyloid interacts with fibrinogen and induces its oligomerization.* Proc Natl Acad Sci U S A, 2010. 107(50): p. 21812-7.
81. Grover, S.P. and N. Mackman, *Intrinsic Pathway of Coagulation and Thrombosis.* Arterioscler Thromb Vasc Biol, 2019. 39(3): p. 331-338.
82. Hofman, Z., et al., *Bradykinin: Inflammatory Product of the Coagulation System.* Clin Rev Allergy Immunol, 2016. 51(2): p. 152-61.
83. Zamolodchikov, D., T. Renne, and S. Strickland, *The Alzheimer's disease peptide beta-amyloid promotes thrombin generation through activation of coagulation factor XII.* J Thromb Haemost, 2016. 14(5): p. 995-1007.
84. Yamamoto-Imoto, H., et al., *A novel detection method of cleaved plasma high-molecular-weight kininogen reveals its correlation with Alzheimer's pathology and cognitive impairment.* Alzheimers Dement (Amst), 2018. 10: p. 480-489.
85. Kononenko, N.L. and M.P. Witter, *Presubiculum layer III conveys retrosplenial input to the medial entorhinal cortex.* Hippocampus, 2012. 22(4): p. 881-95.
86. Passarelli, L., et al., *Uniformity and Diversity of Cortical Projections to Precuneate Areas in the Macaque Monkey: What Defines Area PGm?* Cereb Cortex, 2018. 28(5): p. 1700-1717.
87. Aggleton, J.P., *Understanding retrosplenial amnesia: insights from animal studies.* Neuropsychologia, 2010. 48(8): p. 2328-38.
88. Yamawaki, N., J. Radulovic, and G.M. Shepherd, *A Corticocortical Circuit Directly Links Retrosplenial Cortex to M2 in the Mouse.* J Neurosci, 2016. 36(36): p. 9365-74.
89. Mitchell, A.S., et al., *Retrosplenial cortex and its role in spatial cognition.* Brain Neurosci Adv, 2018. 2: p. 2398212818757098.
90. Powell, A.L., et al., *The retrosplenial cortex and object recency memory in the rat.* Eur J Neurosci, 2017. 45(11): p. 1451-1464.
91. Robinson, S., et al., *Chemogenetic silencing of neurons in retrosplenial cortex disrupts sensory preconditioning.* J Neurosci, 2014. 34(33): p. 10982-8.
92. de Landeta, A.B., et al., *Anterior retrosplenial cortex is required for long-term object recognition memory.* Sci Rep, 2020. 10(1): p. 4002.

93. Nestor, P.J., et al., *Retrosplenial cortex (BA 29/30) hypometabolism in mild cognitive impairment (prodromal Alzheimer's disease).* Eur J Neurosci, 2003. 18(9): p. 2663-7.
94. Pengas, G., et al., *Focal posterior cingulate atrophy in incipient Alzheimer's disease.* Neurobiol Aging, 2010. 31(1): p. 25-33.
95. Poirier, G.L., et al., *Early-onset dysfunction of retrosplenial cortex precedes overt amyloid plaque formation in Tg2576 mice.* Neuroscience, 2011. 174: p. 71-83.
96. Kim, D.H., et al., *Recognition memory impairments and amyloid-beta deposition of the retrosplenial cortex at the early stage of 5XFAD mice.* Physiol Behav, 2020. 222: p. 112891.
97. Govindpani, K., et al., *Vascular Dysfunction in Alzheimer's Disease: A Prelude to the Pathological Process or a Consequence of It?* J Clin Med, 2019. 8(5).
98. Strickland, S., *Blood will out: vascular contributions to Alzheimer's disease.* J Clin Invest, 2018. 128(2): p. 556-563.
99. Cortes-Canteli, M., et al., *Fibrin deposited in the Alzheimer's disease brain promotes neuronal degeneration.* Neurobiol Aging, 2015. 36(2): p. 608-17.
100. Cortes-Canteli, M., et al., *Fibrinogen and altered hemostasis in Alzheimer's disease.* J Alzheimers Dis, 2012. 32(3): p. 599-608.
101. Hultman, K., et al., *Plasmin deficiency leads to fibrin accumulation and a compromised inflammatory response in the mouse brain.* J Thromb Haemost, 2014. 12(5): p. 701-12.
102. Weisel, J.W., *Fibrinogen and fibrin.* Adv Protein Chem, 2005. 70: p. 247-99.
103. Kelly, P., et al., *Restoration of cerebral and systemic microvascular architecture in APP/PS1 transgenic mice following treatment with Liraglutide.* Microcirculation, 2015. 22(2): p. 133-45.
104. Paul, J., S. Strickland, and J.P. Melchor, *Fibrin deposition accelerates neurovascular damage and neuroinflammation in mouse models of Alzheimer's disease.* J Exp Med, 2007. 204(8): p. 1999-2008.
105. Sagare, A.P., et al., *Pericyte loss influences Alzheimer-like neurodegeneration in mice.* Nat Commun, 2013. 4: p. 2932.
106. Sweeney, M.D., A.P. Sagare, and B.V. Zlokovic, *Blood-brain barrier breakdown in Alzheimer disease and other neurodegenerative disorders.* Nat Rev Neurol, 2018. 14(3): p. 133-150.
107. Viggars, A.P., et al., *Alterations in the blood brain barrier in ageing cerebral cortex in relationship to Alzheimer-type pathology: a study in the MRC-CFAS population neuropathology cohort.* Neurosci Lett, 2011. 505(1): p. 25-30.
108. Cullen, K.M., Z. Kocsi, and J. Stone, *Pericapillary haem-rich deposits: evidence for microhaemorrhages in aging human cerebral cortex.* J Cereb Blood Flow Metab, 2005. 25(12): p. 1656-67.
109. Lipinski, B. and E.M. Sajdel-Sulkowska, *New insight into Alzheimer disease: demonstration of fibrin(ogen)-serum albumin insoluble deposits in brain tissue.* Alzheimer Dis Assoc Disord, 2006. 20(4): p. 323-6.
110. Ryu, J.K. and J.G. McLarnon, *A leaky blood-brain barrier, fibrinogen infiltration and microglial reactivity in inflamed Alzheimer's disease brain.* J Cell Mol Med, 2009. 13(9A): p. 2911-25.
111. Davalos, D. and K. Akassoglou, *Fibrinogen as a key regulator of inflammation in disease.* Semin Immunopathol, 2012. 34(1): p. 43-62.

112. Soria, J., et al., *Fibrinogen alphaC domain: Its importance in physiopathology.* Res Pract Thromb Haemost, 2019. 3(2): p. 173-183.
113. Zamolodchikov, D., et al., *Biochemical and structural analysis of the interaction between beta-amyloid and fibrinogen.* Blood, 2016. 128(8): p. 1144-51.
114. Ahn, H.J., et al., *Interactions of beta-amyloid peptide with fibrinogen and coagulation factor XII may contribute to Alzheimer's disease.* Curr Opin Hematol, 2017. 24(5): p. 427-431.
115. Mosesson, M.W., *Fibrinogen and fibrin structure and functions.* J Thromb Haemost, 2005. 3(8): p. 1894-904.
116. Gailani, D. and T. Renne, *The intrinsic pathway of coagulation: a target for treating thromboembolic disease?* J Thromb Haemost, 2007. 5(6): p. 1106-12.
117. O'Brien, R.J. and P.C. Wong, *Amyloid precursor protein processing and Alzheimer's disease.* Annu Rev Neurosci. 34: p. 185-204.
118. Raman, B., et al., *AlphaB-crystallin, a small heat-shock protein, prevents the amyloid fibril growth of an amyloid beta-peptide and beta2-microglobulin.* Biochem J, 2005. 392(Pt 3): p. 573-81.
119. Theriault, P., A. ElAli, and S. Rivest, *High fat diet exacerbates Alzheimer's disease-related pathology in APPswe/PS1 mice.* Oncotarget, 2016. 7(42): p. 67808-67827.
120. Amini, Y., et al., *The Role of Nutrition in Individualized Alzheimer's Risk Reduction.* Curr Nutr Rep, 2020. 9(2): p. 55-63.