High-fat diet increases gliosis and immediate early gene expression in APOE3 mice, but not APOE4 mice

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Abstract

Background: APOE4 is the strongest genetic risk factor for Alzheimer’s disease (AD), and obesity is a strong environmental risk factor for AD. These factors result in multiple central nervous system (CNS) disturbances and significantly increase chances of AD. Since over 20% of the US population carry the APOE4 allele and over 40% are obese, it is important to understand how these risk factors interact to affect neurons and glia in the CNS.

Methods: We fed male and female APOE3 and APOE4 knock-in mice a high-fat diet (HFD-45% kcal fat) or a “control” diet (CD-10% kcal fat) for 12 weeks beginning at 6 months of age. At the end of the 12 weeks, brains were collected and analyzed for gliosis, neuroinflammatory genes, and neuronal integrity.

Results: APOE3 mice on HFD, but not APOE4 mice, experienced increases in gliosis as measured by GFAP and Iba1 immunostaining. APOE4 mice on HFD showed a stronger increase in the expression of Adora2a than APOE3 mice. Finally, APOE3 mice on HFD, but not APOE4 mice, also showed increased neuronal expression of immediate early genes cFos and Arc.

Conclusions: These findings demonstrate that APOE genotype and obesity interact in their effects on important processes particularly related to inflammation and neuronal plasticity in the CNS. During the early stages of obesity, the APOE3 genotype modulates a response to HFD while the APOE4 genotype does not. This supports a model where early dysregulation of inflammation in APOE4 brains could predispose to CNS damages from various insults and later result in the increased CNS damage normally associated with the APOE4 genotype.

Keywords: Obesity, High-fat diet, Apolipoprotein E, APOE, Inflammation, Gliosis

Background

Apolipoprotein E (APOE) 4 is the strongest genetic risk factor for Alzheimer’s disease (AD) [47]. It is present in nearly 25% of the US population and over 50% of AD patients [51]. There are three different APOE alleles: APOE2, APOE3, and APOE4. These alleles encode single amino acid differences at position 112 or 158 [28]. APOE3 is the most common allele and thus APOE3 homozygotes are defined as having an average risk of AD. Meanwhile, APOE4 increases risk of AD: Heterozygous APOE4 carriers are 3-4 times more likely to get AD, while APOE4 homozygous individuals are 15 times more likely [28]. APOE4 can increase the risk of cognitive deficits in healthy individuals and in APOE4 knock-in mouse models [10]. Compared to homozygous APOE3 mice, homozygous APOE4 mice have increased cognitive deficits and decreased neuronal integrity without developing AD pathology [41]. APOE4 mice crossed with mice that exhibit AD pathology have increased plaque accumulation and increased inflammation when compared to homozygous APOE3 mice [42, 49].
Obesity is a strong environmental risk factor for AD [5, 35] and has been reported to affect over 40% of the US population [13]. Obesity is often accompanied by metabolic disturbances such as increases in adipose tissue, hyperglycemia, hypercholesterolemia, insulin resistance, and has increasingly been associated with cognitive deficits [13, 24]. Obesity in rodents can be modeled through high-fat diets (HFD) [25, 27]. Like in humans, rodents on HFD develop increased adipose tissue, glucose intolerance, and insulin resistance [21, 26]. In the central nervous system (CNS), HFD has been associated with increased inflammation (gliosis, cytokine expression) [40, 53] and behavioral deficits (Morris Water Maze), delayed matching, and non-matching task [7, 32].

Here, we investigate how diet induced obesity affects inflammation in the CNS in APOE mice. Since APOE4 is the strongest genetic risk factor for AD and the prevalence of obesity is increasing, it is imperative to understand how the two risks interact and contribute to the eventual inflammatory state of AD. We previously found that HFD induced metabolic syndrome in both APOE3 and APOE4 mice with a stronger effect in APOE4 mice [21]. Here, we have identified the effects of HFD on brain inflammation.

Methods
Animals/diet
Male and female APOE3 and APOE4 knock-in mice, expressing the human APOE allele in the mouse APOE gene locus, on a C57BL/6J background (the gift of Patrick Sullivan) were used. These mice model AD risk without pathological changes. They exhibit normal CNS expression of the APOE gene, but differ in gene expression profiles and behavior across the APOE genotypes [41, 48, 56]. They were fed either a HFD (45% kcal fat, Research Diets-D12451) or ingredient-matched "control" diet (CD) (10% kcal fat, Research Diets-D12450H) for 12 weeks beginning at 6 months of age [21]. HFD fat content was predominantly lard-based, making it high in omega-6 fatty acids (ingredients: 177 g lard, 25 g soybean oil in 858 g of diet), while CD fat content was predominantly soybean oil based making it higher in omega-3 fatty acids (ingredients: 20 g lard, 25 g soybean oil in 1055 g of diet). Food and water were provided ad libitum. At the end of the 12 weeks, mice were euthanized and brains collected for analysis. Mice were euthanized by CO2 inhalation and brains perfused with 1x PBS. Brains of APOE3 and APOE4 mice on CD and HFD (n = 13-15 per genotype and diet, 5-9 per sex) were extracted, and hemi-sections were divided for immunofluorescence (IF) assays, Golgi staining, and biochemical assays. All experiments followed the guidelines of the Georgetown University Institutional Animal Care and Use Committee.

Immunofluorescence
For IF (N = 3-4 per genotype and diet, 1-2 per sex), one brain hemisphere was fixed in 4% PFA and sucrose before freezing in cold 2-methylbutene and slicing at 30 μm. IF stains were performed for Iba1 (Wako, Cat #: 019-19741), GFAP (Cell Signaling, Cat #: 3670S), Doublecortin (DCX, ThermoFisher, Cat #: 481200), NeuN (Chemicon, Cat #: MAB377), and cFOS (Abcam, Cat # ab190289). Stains were imaged using a Zeiss AxioSkop at either 10× (Iba1, GFAP, and cFOS) or 20× (co-stains and DCX) magnification. Staining was analyzed using Image J, with quantification from 4 brain slices/mouse. Hippocampal images were taken from the stratum radiatum and stratum oriens of CA1 and CA3, and the CA4 and the molecular layers (MO) of the dentate gyrus (DG). They were also taken from the arcuate nucleus, the dorsal medial, and lateral hypothalamus, and from multiple layers of the cerebral cortex (Fig. 1). Iba1 and GFAP were analyzed as the percent area covered by IF staining in the image. DCX and cFos expression were analyzed as the counts of antibody positive cells. All quantification was conducted blinded.

Golgi staining
Brain hemissections were placed in Golgi staining solutions according to recommendations (FD Rapid GolgiStain Kit (PK401)) and sliced at 150 μm. Images of the basal shaft (BS) and apical oblique (AO) dendrites of neurons in the entorhinal cortex were acquired with an
Olympus XB51 microscope at 60× magnification with oil immersion. A minimum of 10 neurons were imaged for each mouse (N = 4-5 mice per genotype and diet, 2-3 per sex). Dendritic lengths were measured, and spines counted using Image J. All quantification was conducted in a blinded manner.

RNA analyses
Cortex from one brain hemisphere (N = 5-7 per genotype and diet, 2-4 per sex) was used for RNA isolation. RNA was isolated using the Direct-zol RNA Miniprep kit (Zymo Research R2051) and analyzed by Georgetown University’s Genomic and Epigenomics Shared Resource to determine concentration and purity. For preliminary studies of brain inflammation, equal amounts of four samples for each condition were pooled and analyzed on the NanoString nCounter Mouse Neuroinflammation Panel (Cat #XT-CSO-MNROI1, N = 4 pooled per genotype and diet), which measures transcripts of 770 neuroinflammatory genes. For studies of brain metabolism, three independent samples from each condition were analyzed on the NanoString nCounter Mouse Metabolic Pathways Panel (Cat #XT-CSO-MMP1-12, N = 3 per genotype and diet), which detects transcripts of 768 metabolic genes. All data were analyzed using nSolver 4.0. Background was subtracted from negative controls and samples were normalized to the positive controls and housekeeping genes. Data were further analyzed by normalized count and fold difference, and differences were compared across diet and genotype.

mRNA species that demonstrated at least a twofold difference were subsequently analyzed using qRT-PCR. cDNA (N = 5-7 per genotype and diet, 2-4 per sex) was produced from extracted RNA using the High-Capacity cDNA Reverse Transcription Kit (ThermoFisher Cat #: 4368814). qRT-PCR for Adora2a, Arc, cFos, C3, Egr1, cFos, and TNFα were run with the sequences and temperatures described in Table 1. All qRT-PCR were analyzed in triplicates using the Power SybrGreen Mix (Applied Biosystems) and a 7900HT fast qRT-PCR system. Results were normalized to housekeeping gene GAPDH. Data were analyzed as fold difference compared to CD APOE3 samples as described [29].

Results
HFD increases Iba1 immunoreactivity in APOE3 and APOE4 mice
To examine whether HFD increased microglial activation, we measured Iba1 immunoreactivity in the hippocampus (HPC), cortex (CTX), and hypothalamus (HYP) of APOE3 and APOE4 mice either fed HFD or CD (Fig. 2A). In CA1 of the HPC, HFD APOE3 mice had significantly more Iba1 immunoreactivity when compared to CD APOE3 mice (Fig. 2B, p = 0.035). There were no significant differences between HFD and CD APOE4 mice. Similarly, in CA3 of the HPC, HFD APOE3 mice had significantly more Iba1 immunoreactivity when compared to CD APOE3 mice (Fig. 2C, p = 0.024) and there were no significant differences between HFD and CD APOE4 mice. In the dentate gyrus (DG) of the HPC, there were no significant differences among the groups, although the pattern mirrored CA1 and CA3 results (Fig. 2D). In the CTX and HYP, neither HFD APOE3 nor HFD APOE4 mice had significant differences in Iba1 immunoreactivity when compared to CD mice (Fig. 2E-F). For all areas, there were no statistically significant differences between HFD APOE3 and HFD APOE4 mice or between CD APOE3 and CD APOE4 mice.

HFD increases GFAP immunoreactivity in APOE3 and APOE4 mice
To examine whether HFD increased astrocytic activation, we analyzed GFAP immunofluorescence in the same brain regions (Fig. 3A). In CA1 of the HPC, HFD APOE3 mice trended toward significantly more GFAP immunoreactivity compared to CD APOE3 mice (Fig. 3B, p = 0.045). In CA3 of the HPC, HFD APOE3 mice had significantly more GFAP immunoreactivity (Fig. 3C, p = 0.0009). In the CTX, HFD APOE3 mice had significantly more GFAP immunoreactivity compared to CD APOE3 mice (Fig. 3D, p = 0.0003). In the HYP, neither HFD APOE3 nor HFD APOE4 mice had significant differences in GFAP immunoreactivity when compared to CD mice (Fig. 3E-F). For all areas, there were no statistically significant differences between HFD APOE3 and HFD APOE4 mice or between CD APOE3 and CD APOE4 mice.

Table 1 Primer sequences for qRT-PCR

| Primer | Sequence | Temperature |
|--------|----------|-------------|
| Adora2a Fwd: AGCAACCTGCAAGAAGCTGCACAAC | 60 |
| Arc Fwd: GGAAGGAGGTGCTTCTACGTCG | 55.75 |
| C3 Fwd: GAGGCCCTATGTCCTACCTCCT | 55.75 |
| cFos Fwd: CTTGGGACTCCAAGCTTCAA | 55 |
| Egr1 Fwd: GAGGGAGTATCCCAGCCAA | 57.5 |
| GAPDH Fwd: GTTGTTCCTGTCCTCCGTAGA | 55, 57.5, 60 |
| IL3 Fwd: CTTGGGACTCCAAGCTTCAA | 57.5 |
| Il6 Fwd: AC6GCTTCCTACTGTCCA | 57.5 |
| TNF-a Fwd: GGTGCGATTGTCTCCAGCTT | 60 |
immunoreactivity than CD APOE3 mice (Fig. 3B, \(p = 0.067\)); there was no difference between HFD APOE4 and CD APOE4 mice. In CA3 of the HPC, neither HFD APOE3 nor HFD APOE4 mice had significantly more GFAP immunoreactivity when compared to CD APOE3 mice (Fig. 3C). In the DG of the HPC, HFD APOE3 mice had significantly more GFAP immunoreactivity when compared to CD APOE3 mice (Fig. 3D, \(p = 0.035\)), and there was no significant difference between HFD APOE4 and CD APOE4 mice. In the CTX, HFD APOE3 mice also had significantly more GFAP immunoreactivity than CD APOE3 mice (Fig. 3E, \(p = 0.02\)); again, there was no difference between HFD APOE4 and CD APOE4 mice. In the HYP, there were no significant differences by diet.

**Fig. 2** HFD APOE3 mice, but not APOE4 mice show increased Iba1 immunoreactivity. A Images of Iba1 immunoreactivity in DG of the HPC. B-F Comparison of HFD effects on Iba1 immunoreactivity in CA1, CA3, and DG of the HPC, the CTX, and HYP. E3 CD: light blue, APOE3 mice on a CD, E3 HFD: dark blue, APOE3 mice on a HFD, E4 CD: light red, APOE4 mice on a CD, E4 HFD: dark red. \(N = 3-4\), two-way ANOVA, *\(p < 0.05\)
or genotype (Fig. 3F). When compared across genotypes, there were no differences except for in the CTX where HFD APOE3 mice had significantly more GFAP immunoreactivity than HFD APOE4 mice (Fig. 3E, \( p = 0.009 \)). Thus, HFD was associated with higher measures of gliosis (Iba1 and GFAP) across brain regions in APOE3 mice, but not in APOE4 mice.

**HFD increases immediate early gene expression in APOE3, but not APOE4 mice**

To identify genes related to neuroinflammation or metabolism that may be related to HFD and APOE, we used a NanoString neuroinflammatory panel and a NanoString metabolic panel, and assessed over 1400 genes involved in neuroinflammation and metabolism.
The panels included categories such as adaptive immune response, apoptosis, astrocyte function, inflammatory signaling, innate immune response, lipid metabolism, glucose transport, glycolysis, and fatty acid oxidation and synthesis. The panels also included APOE expression, which did not significantly differ by diet or genotype. From these categories, the two genes that had the highest expression, indicated by the neuroinflammatory panel, and that showed more than a twofold change after HFD were two immediate early gene (IEGs), cFos and Arc. IEGs can be altered by chronic activation of the innate immune system; under inflammatory conditions, increased IEG expression has also been associated with glia-induced increases in neuronal activity [43].

To examine IEG expression across individual brain samples, we used qRT-PCR. Consistent with the pilot data, HFD APOE3 mice had significantly higher cFos gene expression than CD APOE3 mice (Fig. 4A, 66%, \( p = 0.001 \)), while there was no significant difference between HFD APOE4 and CD APOE4 mice. When compared across APOE genotypes, there were no differences in expression between CD APOE3 mice and CD APOE4 mice, but HFD APOE3 mice had significantly higher gene expression than HFD APOE4 mouse (Fig. 4A, \( p = 0.013 \)).

Similar to the pattern of expression of cFos, Arc levels were significantly higher in HFD APOE3 mice compared to CD APOE3 mice (Fig. 4B, 55%, \( p = 0.04 \)); again, there was no significant difference between HFD APOE4 and CD APOE4 mice. When compared across APOE genotypes, HFD APOE3 mice trended toward higher gene expression than HFD APOE4 mice (Fig. 4B, \( p = 0.059 \)), and there were no differences in expression between CD APOE3 mice and CD APOE4 mice. To further test the effects of diet on IEG, we analyzed Erg1, another IEG involved in learning and memory that was not represented on the neuroinflammation panel. Erg1 did not show significant differences between diet or genotype, although its pattern of expression did mirror cFOS and Arc (Fig. 4C).

To analyze IEG protein in the brain across APOE genotypes and diet, we used IF for cFOS and quantified

![Figure 4](https://example.com/fig4.png)

**Fig. 4** HFD APOE3 mice, but not APOE4 mice have increased IEG expression. A Comparison on HFD effects on fold changes in cFOS gene expression. B Comparison on HFD effects on fold changes in Arc gene expression. C Comparison on HFD effects on fold changes in Egr1 gene expression. \( N = 5-6, \) two-way ANOVA, \( *p < 0.05, **p < 0.005 \)
cFOS positive cells in the HPC, CTX, and HYP (Fig. 5A). In CA1 of the HPC, HFD APOE3 mice showed increased cFOS-positive cells when compared to CD APOE3 mice (Fig. 5B, $p = 0.03$), consistent with the mRNA data. There was no difference between HFD APOE4 and CD APOE4 mice and no differences when compared across genotypes. In CA3, HFD APOE3 mice trended toward more cFOS positive cells when compared to CD APOE3 mice (Fig. 5C, $p = 0.082$).

**Fig. 5** HFD APOE3 mice, but not APOE4 mice have increased cFOS immunoreactivity. **A** Image of cFOS positive cells in the DG. **B-F** Quantification of cFOS positive cells in CA1, CA3, and DG of the HPC, the CTX, and HYP. **G** Representative image of cFOS positive cells colocalized with neurons. $N = 3-4$, two-way ANOVA, *$p < 0.05$
HFD differentially affects other neuroinflammatory genes in APOE3 and APOE4 mice

Along with IEGs, the NanoString nCounter mRNA analysis panel indicated changes in several other genes related to neuroinflammation: Adora2a, C3, and IL-3. Adora2a is an anti-inflammatory gene with properties protective against tissue damage [37, 50]. HFD APOE4 mice exhibited significantly higher levels of Adora2a expression than CD APOE4 mice both by NanoString nCounter analysis (Fig. 6A, p = 0.002) and qRT-PCR analysis (Fig. 6B, p = 0.046). There were no differences between HFD and CD APOE3 mice. When compared across APOE genotypes, there were no significant differences in Adora2a expression between mice with the NanoString nCounter analysis, but with qRT-PCR, CD APOE3 mice had significantly higher gene expression than CD APOE4 mice (Fig. 6B, p = 0.029). There were no differences between HFD APOE3 and HFD APOE4 mice. Thus, HFD increased Adora2a expression, particularly in the APOE4 mice.

The remaining genes indicated by the neuroinflammatory panel were only analyzed by qRT-PCR because of the lower mRNA expression levels. The complement factor C3 plays an important role in innate immunity. HFD APOE3 mice exhibited significantly higher levels of C3 gene expression than CD APOE3 mice (Fig. 6C, p = 0.032). There were no differences between HFD and CD APOE4 mice and no differences by APOE genotype. To examine C3 activity, we ran a western blot and examined cleaved C3; however, we did not find any difference in C3 or cleaved C3 levels (data not shown). For the other neuroinflammatory gene identified (IL-3), as well as the common markers of general inflammation TNF-α and IL-6, there were no differences by diet or genotype (Fig. 6D-F).

HFD does not alter neurogenesis or spine density in APOE3 and APOE4 mice

Neuronal degeneration has been associated with obesity and inflammation [3]. To test whether HFD here affected neuronal integrity, we examined spine density and hippocampal neurogenesis (Fig. 7A-B). For spine density, Golgi-stained pyramidal neurons in the entorhinal cortex were analyzed for dendritic spine density, quantified on the apical oblique and basal shaft dendrites. There were no significant differences in spine density across genotypes or diets (Fig. 7C-D). To examine whether HFD affects neurogenesis, we analyzed neurons in the DG of the HPC by DCX immunostaining. There were no significant differences in neurogenesis across genotypes or diets (Fig. 7E).

Discussion

It is important to understand how environmental factors combine with genetic factors to affect inflammation and alter the risk of AD. Here, we focused on the rapidly growing environmental risk factor, obesity, and the strongest genetic risk factor, APOE4, and sought to understand how the combination affects inflammation. Using a mouse model of the human APOE alleles, we found that HFD induced increased microglia and astrocyte expression in APOE3, but not APOE4 brains. This same pattern continued with increases in the IEGs cFOS and Arc in APOE3, but not APOE4 brains. These APOE-related CNS effects occur along with multiple metabolic disturbances: weight gain, adipose tissue accumulation, and glucose intolerance. These peripheral changes occurred predominantly in APOE4 mice; no behavioral changes were seen as a result of the HFD [21].

Previously HFD has been associated with increases in both microglial and astrocytic activity in wild-type mice [40, 53]. The APOE mice in our study are similar to control mice in that they do not have accumulations of Aβ or phospho-tau. Furthermore, the APOE3 mice act as controls and perform similarly to wild-type mice. Consistent with the study of wild-type mice, APOE3 mice in our study showed an increase in microgliosis and astrogliosis, although APOE4 mice did not. Another study showed HFD caused a decrease in CD68 (a marker of monocyte-derived cells) in APOE4 mice [17]. While data support that APOE4 mice have an increased response to short-term noxious stimuli such as LPS treatment or injury [23, 52, 57], there is evidence of a decreased response to the chronic inflammation caused by obesity [8, 17]. In one study, APOE3 and APOE4 mice were fed a HFD for approximately 7 months, and there were APOE3-specific increases in expression of TNFα, IL-6, and CD36 when compared to APOE4 mice [8]. APOE4 mice also exhibited lower expression of LPS immune sensors indicating a decreased ability for innate immune detection [8]. We propose that APOE3 mice have wild-type responses to chronic inflammatory conditions, while APOE4 mice may more readily respond to specific acute stimuli but have a more muted response to chronic stimuli.

Several studies have addressed the effects of HFD in AD mouse models related to APOE. HFD increases both Aβ and phospho-tau pathology across AD mouse models [22, 27]; similar effects are seen in AD-APOE
mouse models, but the responses differ by APOE genotype. In EFAD mice (mice that possess both a human APOE genotype and 5xFAD transgenes) on a HFD, there was an increase in gliosis and AD pathology in E4FAD mice, but not in E3FAD mice [34]. In APP/APOE mice (APOE mice crossed with APP/PS1ΔE9), there was an increase in AD pathology in APOE4, but not APOE3 mice on HFD [36]. The HFD may be affecting an already compromised inflammatory system responding to the increased amyloid found in the APOE4 mice.

We propose that, while chronic inflammation is damaging, inflammation at an early stage of obesity may be neuroprotective, and generally not experienced in APOE4 mice. Inflammation is associated with neuroprotection in early disease stages [15, 45] and the response in the APOE3 mice could be indicative of a similar...
mechanism. In AD mouse models, APOE3 has been associated with increases in microglial-plaque interactions when compared to APOE4 mice [46], an example of a positive inflammatory response in the APOE3 CNS (although there might be differences based on plaque morphology [42]). The inflammatory response from APOE3 mice can be beneficial for Aβ clearance and, without it, the APOE4 mice may suffer.

In addition to inflammatory processes, glia are also heavily involved in lipid metabolism. Increased gliosis in the APOE3 mice may be a response to increased lipids and processing of lipids. Fatty acids can cross the blood-brain barrier and be taken up and stored by astrocytes; this ability prevents lipid induced neuronal damage [4]. Microglia also respond to increases in lipids and, when healthy, assist in the clearance of excess lipids [30]. The measures of astrogliosis and microgliosis in APOE3 brains could reflect changes to increased CNS lipids, and the APOE4 mice may have a deficit in responding to lipid-related stressors.

One gene significantly altered by diet and APOE genotype in the NanoString metabolic panel was Adora2a.
(also known as the adenosine A2A receptor), which is associated with inflammation [11]. While the panel had multiple metabolic categories, none of the genes specifically associated with metabolism were altered. Adora2a is expressed in the cortex and hippocampus; after CNS damage, there is an increase of Adora2a expression on glia, which is associated with increased activity and inflammation [6]. In multiple neurodegenerative conditions, there is an increase in Adora2a resulting in neuronal excitotoxicity and cell death [6]. Here, we see an increase in Adora2a in APOE4 mice on HFD, indicating potential enhancement in glutamatergic activity [11], which could lead to neuronal damage if prolonged. While the APOE4 mice do not demonstrate increased gliosis at this stage of developing obesity, there could be stronger inflammation and neuronal damage if increased Adora2a expression persisted. An APOE3-specific response to HFD was further emphasized through expression of IEGs indicated by the NanoString neuroinflammatory panel and confirmed by qRT-PCR. We found that HFD increased cFOS and Arc expression twofold in APOE3 mice, with no effects in APOE4 mice. This expression was observed in neurons, with the counts of cFOS-positive neurons reproducing the effects of HFD on mRNA measures. IEGs are genes that are often rapidly and transiently expressed as direct responses to stimuli such as novel environments or injury. IEG expression is also associated with alterations in synaptic plasticity, with either up- or downregulation of IEGs being associated with increased and decreased LTP and memory retention, respectively [1, 12, 33]. Changes in IEG activity are also associated with behavioral tasks such as novel object recognition, and fear conditioning, indicative of their role in learning [33].

IEG expression also changes from neuronal stress or damage. IEG expression is increased in AD brain [31]. These increases have been linked to more GFAP positive astrocytes and thioflavin-stained plaques [2] and in Aβ-mediated apoptosis [31]. There are also alterations in neuronal IEG expression after traumatic brain injuries [14, 38] and after chronic LPS-induced inflammation [43, 44]. After chronic LPS infusion, mice were tested on a memory paradigm and those treated with LPS had increased IEG expression. This increased IEG expression was directly correlated with increased microglia activity [44]. Overall, the chronic inflammation led to gliosis and that resulted in increased IEG activity and cognitive deficits [44]. We propose a similar process in our study, with HFD, in the APOE3 but not APOE4 mice, increasing gliosis and that gliosis is leading to altered IEG expression.

In many of the published studies, IEG expression was measured after behavioral stimulations such as learning and memory challenges. Here, we have not directly induced IEG expression, suggesting another mechanism. There may be APOE3 specific neuronal adaptations to HFD expressed through IEG activity. IEG increases have been noted without additional stimuli in the HYP after an extended period on HFD, resulting in adipose tissue storage [54]. IEG activity also acts as an indicator of neuronal activity resulting in long-term adaptations within neural circuits [55]. The increased IEG expression we are seeing could affect the processing and storage of specific lipid components of the HFD, and that could lead to increased protection in APOE3 mice. Lack of HFD-induced IEG activity, as in the APOE4 mice, could lead to the more detrimental outcomes. The NanoString neuroinflammatory panel only indicated robust changes in the IEGs in APOE3 mice on HFD. There was no indication of more severe neuroinflammation, further emphasizing that the mice are not currently suffering from detrimental neuroinflammation. Rather, the APOE3 mice may be exhibiting compensational neuronal adaptations in response to HFD.

The APOE3 mice exhibited several CNS alterations associated with HFD that the APOE4 mice did not. These APOE3 mice also showed decreased metabolic disturbances compared to APOE4 mice [21]. We propose that during HFD, APOE3 mice are able to induce protective inflammatory pathways that have positive peripheral and CNS effects. These mice were 10 months old at the end of all experimentation and were on HFD for a total of 4 months; therefore, we may be seeing the preliminary effects of HFD as they continue to gain weight. Prolonged obesity could result in more detrimental effects such as increased noxious inflammation, neuronal damage, and behavioral deficits. APOE4 mice on HFD for extended periods or on diets with higher fat contents exhibit increased inflammation and behavioral deficits [18–20].

The APOE4 allele is the ancestral version with the APOE3 allele potentially evolving as a result in shifts in diet availability [9, 16, 39]. Evolutionarily, APOE3 may allow humans to compensate for increased diet availability more easily and to process dietary fats, while APOE4 remains better suited for environments where diet availability is scarce.

**Conclusion**

Overall, we observed increases in HFD-induced gliosis and IEG expression specific to the mice expressing APOE3 and an increase in Adora2a expression in mice expressing APOE4. These changes were apparent without robust changes in an array of other inflammatory genes. This work suggests that initial gliosis and IEGs may perform a novel protective role in response to early stages of obesity. More broadly, this approach of combining strong genetic and environmental factors is necessary for providing insight into personal risks of
important CNS impairment and potential for AD development.

Limitations

While these data identify specific CNS alterations in APOE3 mice that do not occur in APOE4, the study has important limitations. It does not identify any specific mechanisms that lead to the CNS alterations caused by HFD, or the downstream consequences of those alterations. In addition, the conclusions about HFD inducing gliosis in APOE3 mice are based on small sample numbers, with only three to four animals (of mixed sex) per group. For qRT-PCR analyses of the identified genes, we examined two to three animals per sex per group, and we did not observe any trends suggesting differences by sex. Given the importance of sex in the risk of AD, further studies could be powered to examine the combined effects of APOE genotype, and diet. Sex. Finally, while we observed strong differences in gliosis in the HPC, a larger sample size may have allowed us to better test for significant differences in the other areas.

Abbreviations

AD: Alzheimer’s disease; AO: Apical oblique; APOE: Apolipoprotein E; BS: Basal shaft; CNS: Central nervous system; CTX: Cortex; DCX: Doublecortin; HFD: High-fat diet; HPC: Hippocampus; IEG: Immediate early gene; LTP: Long-term potentiation

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Authors’ contributions

NJ designed, analyzed, and interpreted the data. NJ also drafted and revised the work. KW analyzed the data. GWR supervised the work. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

All experiments followed the guidelines of the Institutional Animal Care and Use Committee.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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