eNeuro

Research Article: New Research | Disorders of the Nervous System

Metabolic disturbances of a high fat diet are dependent on APOE genotype and sex

https://doi.org/10.1523/ENEURO.0267-19.2019

Cite as: eNeuro 2019; 10.1523/ENEURO.0267-19.2019

Received: 9 July 2019
Revised: 3 September 2019
Accepted: 18 September 2019

1. **Manuscript Title**: Metabolic disturbances of a high fat diet are dependent on APOE genotype and sex.
2. **Abbreviated Title:** HFD disturbances depend on APOE genotype and sex
3. **Authors:** Nahdia S. Jones, Katarina Q. Watson, G. William Rebeck, **Affiliations:** Department of Neuroscience, Georgetown University, Washington, DC 20007
4. **Author Contributions:** Nahdia S. Jones- Designed research, Performed research, Analyzed data, Wrote the paper. Katarina Q. Watson- Analyzed data, Wrote the paper. G. William Rebeck- Designed research, Wrote the paper.
5. **Corresponding Author:** G. William Rebeck, gwr2@georgetown.edu, 3970 Reservoir Rd NW, Washington, DC 20007
6. **Number of Figures:** 7
7. **Word Count Abstract:** 174
8. **Word Count Significant Statement:** 89
9. **Word Count Introduction:** 523
10. **Word Count Discussion:** 1423
11. **Acknowledgements:** This work was funded by NIH R01 NS100704 and NS100704-S1. We would like to thank the Georgetown Preclinical Imaging Lab for assistance in experimental design and image analysis.
12. **Conflict of Interest:** The authors declare no competing financial interests.
13. **Funding Source:** NIH R01 NS100704 and NS100704-S1

Abstract

Objective: Apolipoprotein E4 (*APOE4*) is the strongest genetic risk factor for Alzheimer's Disease (AD). *APOE4* is also associated with an increased risk of metabolic syndrome. Obesity is a major environmental risk factor for AD. While *APOE* genotype and obesity independently affect metabolism and cognition, they may also have synergistic effects. Here we examined the metabolic and behavioral alterations associated with a high fat diet (HFD) in male and female *APOE* knock-in mice.

Methods: Male and female mice were fed a 45% kcal HFD or a 10% kcal low fat diet (LFD) for 12 weeks and adipose tissue accumulation, glucose levels, anxiety-like behavior, and spatial memory were examined.

Results: We found that with HFD, male *APOE4* mice were more susceptible to metabolic disturbances, including visceral adipose tissue accumulation and glucose intolerance when compared to *APOE3* mice, while female *APOE3* and *APOE4* mice had similar metabolic responses. Behaviorally, there were no effects of HFD in mice of either genotype.

Conclusions: Our results suggest that metabolic responses to HFD are dependent on both sex and APOE genotype.

Significance Statement

APOE4 and obesity are independently associated with increased risk of metabolic syndrome and cognitive impairment. Obesity may cause greater metabolic and cognitive disturbances in *APOE4* carriers. However, the metabolic and cognitive effects of obesity on male and female *APOE4* carriers remain unknown. Here we examine and compare the metabolic and cognitive disturbances caused by a high fat diet in both male and female *APOE3* and *APOE4* mice. Through this study, we examine how high fat diet affects the *APOE3* and *APOE4* genotype and how these effects differ across sexes.

Introduction

Apolipoprotein E4 (*APOE4*) is the strongest genetic risk factor for Alzheimer's Disease (AD) (Huang, Weisgraber et al. 2004, Raber, Huang et al. 2004). In the periphery, APOE is a component of lipoproteins responsible for the metabolism of plasma lipids. Through binding to different lipoprotein receptors, APOE traffics high density lipoproteins (HDLs) and very low density lipoproteins (VLDLs) throughout the body for storage or elimination (Huang and Mahley 2014). In the central nervous system (CNS), APOE-HDL are responsible for trafficking lipids from astrocytes to neurons and for clearance into the circulation (Liu, Liu et al. 2013). There are three *APOE* alleles, *APOE2*, *APOE3*, and *APOE4*, and each allele is associated with a differential risk of AD. *APOE2* has an allele frequency of 8% in the US and is associated with a 40% decreased risk of developing AD (Huang and Mahley 2014). *APOE3* has an allele frequency of 77%; homozygous *APOE3* carriers (64% of the population) are defined as having a normal risk of AD (Liu, Liu et al. 2013). *APOE4* has an allele frequency of 15%; heterozygous carriers are 2.3 times more likely to develop AD and homozygous carriers are 14 times more likely (Liu, Liu et al. 2013).

Obesity and metabolic syndrome are also major risk factors for AD. Obesity is a medical condition characterized by increased body mass index and currently affects 40% of adults and 20% of children in the US (Hales, Carroll et al. 2017). In the periphery, obesity can lead to metabolic syndrome including increases in visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT), and decreases in glucose metabolism and insulin sensitivity (Mathieu, Poirier et al. 2009, Neth and Craft 2017). In the CNS, obesity is associated with increased inflammation, deficits in cognitive functioning, mild cognitive impairment, and AD (Gustafson, Backman et al. 2009, Besser, Gill et al. 2014, Bloor and Symonds 2014).

While *APOE* genotype and obesity independently affect AD risk, they may also have combined effects. *APOE4* is associated with increased cognitive deficits and increased risk of metabolic syndrome (Arbones-Mainar, Johnson et al. 2008, Rodriguez, Burns et al. 2013, Torres-Perez, Ledesma et al. 2016), which are exacerbated when combined with obesity. Obese *APOE4* carriers can have elevated glucose and insulin levels (Elosua, Demissie et al. 2003), and deficits in cognitive functioning (Ghebranious, Mukesh et al. 2011, Zade, Beiser et al. 2013). Data in humans is complemented by mouse models. *APOE4* knock-in mice have increased insulin resistance and deficits in glucose metabolism when on high fat diets (Arbones-Mainar, Johnson et al. 2008, Johnson, Torres et al. 2017). Cognitive performance of *APOE4* mice on high fat diets have shown mixed results, with either increased deficits in spatial memory (Johnson, Torres et al. 2017) or no cognitive differences (Janssen, Jansen et al. 2016). Here we compare the effects of a high fat diet (HFD), with macronutrients equivalent to a western diet, on male and female homozygous *APOE3* and *APOE4* mice. We examined both metabolic and behavioral alterations and found that HFD increases metabolic disturbances in both *APOE3* and *APOE4* mice, with *APOE4* mice being more robustly affected. We also found that male and female mice differentially respond to HFD.

Methods

Animals/Diet

Male and female human *APOE3* and *APOE4* knock-in mice on a C57BL/6J background (n=5-9/sex) (the gift of Patrick Sullivan) were fed either a HFD (45%kcal fat, Research Diets-D12451) or ingredient matched low fat diet (LFD) (10%kcal fat, Research Diets-D12450H) for 12 weeks

beginning at 6 months of age. Food and water were provided ad libitum and mice were weighed weekly during the 12 weeks. At the end of the 12 weeks mice underwent glucose tolerance testing (GTT), abdominal and neck magnetic resonance imaging (MRI), and behavioral assays which occurred over a two-week period (12-14 weeks). The mice remained on the diets throughout the GTT, MRIs, and behavioral assays. All experiments followed the guidelines of the Institutional Animal Care and Use Committee.

Glucose Testing

Mice were restricted from food for 6 hours prior to the measures of baseline glucose levels and glucose tolerance to a glucose bolus. Fasting baseline glucose levels were followed by an intraperitoneal injection of 20% glucose (1mg/kg). Blood glucose levels from tail vein withdrawal were measured using the AccuChek Guide glucose meter at 15, 30, 60, and 120 minutes post injection.

Magnetic Resonance Imaging

After completion of behavioral assays, mice underwent small animal imaging in the Preclinical Imaging Research Laboratory at the Georgetown University Medical Center. Mice were anesthetized using 3-5% isoflurane and maintained with 1-3% isoflurane. Images were taken with a 7-Tesla horizontal Bruker spectrometer ran by Paravision 5.1; body temperature, heart rate, and respiration were monitored throughout the scan. Images were obtained for the abdominal while adipose tissues (WAT) visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT), and for the neck brown adipose tissue (BAT). Z stack images were analyzed with ImageJ. VAT and SAT images were quantified as ratios of abdominal adipose tissue to abdominal organs (referred to as “body”). For BAT, images were quantified as the ratio of BAT intensity to the white adipose

tissues (WAT) intensity. The BAT and WAT intensities were measured using the mean grey value in ImageJ, with the darker areas being reflected as higher mean grey values indication higher BAT intensities. Higher BAT intensity indicates more BAT, which has the ability to convert excess food energy into thermal energy (Schulz and Tseng 2013).

Behavioral Assays: Open Field, Elevated Zero, Barnes Maze

For all behavioral assays, mice were placed in the behavioral suite for a 30-minute acclimation period.

Open Field Test

Mice freely explored a square (43 x 43 x 30 cm) open field apparatus for 300 seconds. During free exploration, locomotor activity and anxiety-like behaviors were recorded. The apparatus was divided into an inner zone and a bordering outer zone that lined the apparatus's walls. Mice were placed in the center of the inner zone and behavior was recorded for the duration of the test. Behavior was recorded with Med Associates Activity Monitor 7. For locomotion, average speed (m/s) was assessed. For anxiety-like behavior, time spent in the inner vs outer zone (9 x 9 cm) was assessed as increased time in the outer zone indicating increased anxiety (Seibenhener and Wooten 2015). Data were analyzed with GraphPad Prism 8.

Elevated Zero Maze

Mice were exposed to a circular elevated zero apparatus (50cm from floor, 50cm diameter, 15cm high closed regions) for 300 seconds of free exploration. The apparatus consists two closed regions and two open regions of equal sizes. Mice were placed on the center of an open region to begin testing and behavior was recorded for the duration of the 300 seconds using ANY-maze Behavioral

tracking software 6.0. Time spent in the closed vs open regions of the apparatus was examined as a measure of anxiety and willingness to explore. Data were analyzed with GraphPad Prism 8.

Barnes Maze

Mice were exposed to the Barnes maze for five consecutive days to test spatial learning and memory, as described (Speidell, Demby et al. 2019). The maze was present in a brightly lit room and 90dB white noise. Mice were habituated to the maze on day one, and then had four consecutive training days. During training days, mice underwent four trials with 15 minutes between each trial, and latency to first nose poke and latency to enter the escape hole (latency to escape) were recorded to examine spatial memory. Mice were recorded with Any-maze Behavioral tracking software 6.0 and data were analyzed with GraphPad Prism 8.

Statistics

All data are expressed as mean ± standard deviation with the exception of behavioral assays which are expressed as mean ± standard error. Comparisons between genotype, sex and diet were analyzed by three-way ANOVAs with Tukey's multiple comparison test. Comparisons between genotype and sex were analyzed by two-way ANOVAs with Sidak's multiple comparison test. Statistical significance was determined by a probability error of $p<0.05$. All analyses were done using GraphPad Prism 8.

Results

High fat diet increases the weight of *APOE3* and *APOE4* mice

159 To examine how the different *APOE* genotypes respond to obesity, we used a diet induced
160 obesity model. Male and female mice (6 months old) were placed on a HFD for 12 weeks and
161 weighed weekly. At 6 months old, the male mice (across genotypes) weighed significantly more
162 than female mice ($p<0.0001$, Figure 1A). To directly compare weight gain trajectories, weights
163 were calculated as a percent of each mouse's original body weight. All groups gained weight over
164 the course of the experiment, with HFD groups gaining more weight compared to LFD groups
165 (Figure 1).

166 In female mice, HFD resulted in a 40% increase from original body weight by week 11 in
167 both *APOE3* and *APOE4* mice ($p<.0001$); both genotypes gained weight at the same rate. HFD
168 mice also weighed more than LFD mice (*APOE3*: $p<.06$, *APOE4*: $p<.005$). Female *APOE3* and
169 *APOE4* mice on the LFD experienced slight weight gain (15%, $p>0.8$, Figure 1B).

170 In male mice, HFD resulted in a 30% increase from original body weight in *APOE3* mice
171 by week 11 ($p<.0001$) and resulted in a 45% increase from original body weight in *APOE4* mice
172 by week 11 ($p<.0001$), although the differences between *APOE3* and *APOE4* genotypes were not
173 statistically significant ($p=0.15$). *APOE3* and *APOE4* mice on the LFD experienced slight weight
174 gain (17%, $p>0.8$, Figure 1C). HFD *APOE3* mice did not weigh significantly more than the LFD
175 *APOE3* mice ($p=.5697$); HFD *APOE4* mice did weigh significantly more than LFD *APOE4* mice
176 ($p<.0001$).

177 Across sexes, the weight gain due to HFD did not differ. However, while male *APOE3* did
178 respond to HFD, they gained 15% less weight than male *APOE4* mice or female mice (Figure 1D).
179 On week 12, there was a slight decrease in body weight associated with the beginning of the
180 metabolic and behavioral assays; therefore, statistical tests were conducted on data from week 11.

HFD increases baseline glucose levels and glucose intolerance in *APOE3* and *APOE4* mice

Deficits in glucose metabolism are also associated with HFD. These deficits can lead to Type II Diabetes and cognitive deficits. To test whether our model alters glucose metabolism, baseline glucose levels and glucose tolerance were measured after 12 weeks of HFD (Figure 2).

In females, HFD *APOE3* and *APOE4* mice had similar baseline glucose levels; these levels did not differ from LFD *APOE3* and *APOE4* mice. In males, HFD *APOE3* and *APOE4* mice had similar baseline glucose levels; however, their levels were elevated when compared to LFD *APOE3* and *APOE4* mice ($p<.002$, Figure 2A). Across sexes, male HFD mice had significantly higher baseline glucose levels than the female HFD mice ($p<.0001$, Figure 2A). We reasoned that increased baseline glucose may be associated with weight gain, given the disparate levels of weight gains in male versus female mice. To test this hypothesis, we determined the correlation of weight gain with baseline glucose across genotype and sex. *APOE3* and *APOE4* weight gain was positively correlated with increased baseline glucose in males, but not females (APOE3: $p=0.001$, $R2=0.68$, APOE4: $p=0.003$, $R2=.62$, Figure 2B).

After baseline glucose levels, mice underwent GTT as a measurement of glucose metabolism. A bolus of glucose was given, and glucose levels measured at 15, 30, 60 and 120 minutes. In females, when compared to baseline, there was an increase in glucose levels in the first 15 minutes in all groups ($p<.003$). This increase remained in the HFD groups at 30 minutes ($p<.0001$), and 60 minutes ($p<.0001$, Figure 2C). In males, when compared to baseline, there was an increase in glucose levels in the first 15 minutes and remained elevated at 30 minutes in all groups ($p<.02$). At 60 minutes, all mice returned to the range of baseline glucose except for HFD *APOE4* mice ($p<.002$, Figure 2D). This indicates that the HFD *APOE4* mice did not metabolize the glucose as quickly or efficiently as the HFD *APOE3* mice or the LFD *APOE4* mice.

To examine overall differences in glucose tolerance over time across genotype and sex, we analyzed area under the curve in the GTT. In females, HFD *APOE4* mice had a larger deviation in glucose than HFD *APOE3* mice ($p<.0003$) and LFD *APOE4* mice had a larger deviation in glucose than LFD *APOE3* mice ($p<.02$). HFD *APOE4* mice also had a larger deviation than LFD *APOE4* mice ($p<.02$). This difference was not seen when comparing HFD *APOE3* mice and LFD *APOE3* mice (Figure 2E). In males, HFD *APOE4* mice had a larger deviation in glucose than HFD *APOE3* mice ($p<.0001$). HFD mice also had a larger deviation in glucose than both LFD *APOE*3 mice ($p<.05$), and *APOE4* mice ($p<.0001$; Figure 2E). Across sexes, glucose deviations in male HFD *APOE4* mice were greater than deviations seen in all female groups ($p<.0003$). Deviations in male HFD groups were larger than deviations seen in the female HFD groups except female HFD *APOE4* mice ($p<.003$, Figure 2E).

To test whether the glucose intolerances found could result from weight gains, we ran correlational analyses comparing weight gain with glucose levels 30 minutes after bolus. In both *APOE3* and *APOE4* mice, an increase in weight was positively correlated with higher glucose levels ($p<.007$), indicating any increase in weight may strongly affect glucose intolerance. There was a stronger positive correlation between weight gain and glucose intolerance in *APOE3* mice ($p=0.01$) indicating weigh gain can drive glucose intolerance in *APOE3* mice while *APOE4* mice are more susceptible to glucose intolerance at lower weights (Figure 2F). Glucose levels were significantly correlated with weight gain regardless of sex with the exception of *APOE4* males ($p<.02$, Figure 2F).

HFD increases visceral adipose tissue and subcutaneous adipose tissue in *APOE3* and *APOE4* mice

A metabolic disturbance associated with HFD is increased adipose tissue. SAT is the adipose tissue more associated with obesity; however, VAT is more noxious due to its direct contact with the organs and its ability to release inflammatory cytokines (Hotamisligil, Arner et al. 1995). To test whether our model results in increases in specific types of adipose tissue, we used small rodent MRIs to examine both VAT and SAT levels (Figure 3A).

In females, HFD caused an increase in VAT compared to LFD ($p<.0001$, Figure 3B). In males, HFD *APOE4* mice accumulated more VAT than LFD *APOE4* mice ($p<.0001$), but there was no similar effect for *APOE3* mice ($p=1.0$, Figure 3B). Across sexes, HFD mice had similar elevated VAT levels, except for the male *APOE3* mice, which did not differ from LFD mice ($p<.02$, Figure 3B).

We analyzed the correlation between VAT and GTT, including the possible effects of genotype, sex, and diet. There was no correlation between VAT and GTT when considering genotype and diet (Figure 3C); however, there was a correlation between VAT and GTT only in male *APOE4* mice ($R^2=0.6$, $p=0.03$, Figure 3C). We also ran correlational analyses comparing weight gain and VAT to see whether VAT was a large contributor to the weight gain. VAT and weight gain in *APOE3* females and *APOE4* mice positively correlated (*APOE3*: $R^2=0.52$ $p=0.005$, *APOE4*: $R^2=0.40$, $p=0.01$); however, there was not a positive correlation between VAT and weight gain in *APOE3* males (Figure 3D). These findings indicate that VAT may act as a contributor to weight gain and glucose intolerance.

The effects of HFD on SAT mirrored its effects on VAT. In females, HFD *APOE3* and *APOE4* mice had similar levels of SAT, and HFD caused an increase in SAT compared to LFD. ($p<.03$). In males, SAT accumulation did not differ across genotype or diet (Figure 4A). VAT and SAT levels strongly correlated ($R^2=0.47$, $p<.0001$, Figure 4B).

Sex affects BAT area and intensity in APOE3 and APOE4 mice

BAT is a metabolically active adipose tissue (Schulz and Tseng 2013). To examine diet associated BAT alterations, we used small rodent MRI and imaged neck BAT (Figure 5A). We examined intensity of BAT, with decreasing intensities indicating the transition to WAT. There was no effect of diet on BAT; however, there were sex differences. Male mice had significantly lower BAT intensities than female mice (~30%, $p<.004$, Figure 5B). The lower BAT intensities indicate less thermogenic energy expenditure which has been implicated in decreased resistance to diet induced obesity (Schulz and Tseng 2013). We ran correlational analyses to see whether BAT intensity individually correlated with weight gain. In male *APOE4* mice, there was a negative correlation between weight gain and BAT intensity ($R^2=0.42$, $p=0.01$, Figure 5C). This was also seen in *APOE3* mice ($R^2=0.3$, $p=0.03$, Figure 5C). These correlations indicate weight gain can directly decrease BAT levels, particularly in *APOE3* and male *APOE4* mice.

Effects of HFD on behavior in *APOE3* and *APOE4* mice

We tested the effects of *APOE* genotype, sex, and diet on metabolism affected cognitive domains in these mice. Since HFD resulted in significant weight gain, we first examined whether movement had been impaired. In the open field test, there were no differences in average speed regardless of diet, sex, or genotype (Figure 6A).

To examine whether HFD induced cognitive alterations in this experiment, we used the open field test (OFT), elevated zero maze (EZM), and Barnes maze (BM). OFT and EZM were both used to measure anxiety like behavior. For the OFT, time spent in the center zone was used as a measure of decreased anxiety. We found that all *APOE4* mice spend less time in the center zone than HFD *APOE3* mice (*$p<.05$, **$p<.002$, Figure 6B). There were no differences between

sexes (data not shown). EZM, a second measure of anxiety like behavior, did not show any differences by diet, *APOE* genotype, or sex (Figure 6C).

We used the BM to test spatial learning and memory. The mice were exposed to the maze for four training days and latency to first nose poke and latency to escape were measured each day. For latency to first nose poke *APOE4* mice showed less learning on training day one, but matched *APOE3* mice by training day two ($p<.03$, Figure 7A). For latency to escape, *APOE4* mice were delayed for the first two training days, but by training day three the latency to escape matched *APOE3* mice. There was no effect of diet on either *APOE3* or *APOE4* groups ($p<.03$, Figure 7B).

Discussion

Although not as severe as the risk of AD in homozygous *APOE4* carriers, metabolic disturbances caused by a high fat diet can have a 2-3 fold increased risk of cognitive impairment and Alzheimer's Disease (Gunstad, Paul et al. 2007, Whitmer, Gustafson et al. 2008, Profenno, Porsteinsson et al. 2010). Using an *APOE* knock-in mouse model, we found that *APOE4* increases metabolic disturbances in response to HFD. Furthermore, sex plays a pivotal role in the effects of HFD. We examined differences in weight, baseline glucose levels, glucose intolerance, and adipose tissue disposition and found these to be the most significantly increased in male *APOE4* mice. Female *APOE3* and *APOE4* mice responded similarly to HFD with increased weight, glucose intolerance, and adipose tissue levels. In terms of the types of adipose tissue that increased under the HFD, in males, VAT increases were seen in the *APOE4* mice, but not *APOE3* mice. SAT increases were not seen in *APOE3* or *APOE4* mice. These findings demonstrate that the male *APOE4* group has the greatest accumulation of VAT in response to HFD. In females, VAT and

293 SAT increases were seen in both *APOE3* and *APOE4* mice in response to HFD, indicating there is
294 a similar accumulation in both types of adipose tissue. Throughout the study, female mice had
295 similar metabolic responses to HFD regardless of *APOE* genotype and male *APOE4* mice had
296 more robust metabolic disturbances.

297 While we cannot directly compare our study to previous studies due differences in the age
298 of mice, diet composition, and length of time on diets, there are similarities across models. With
299 wild-type mice on HFD, male and female mice accumulate similar levels of VAT, but male mice
300 display higher fasting blood glucose levels, insulin levels, and insulin resistance (Macotela,
301 Boucher et al. 2009, Hwang, Wang et al. 2010, Medrikova, Jilkova et al. 2012, Barron, Rosario et
302 al. 2013). Human studies also showed this pattern: males have increased chances of metabolic
303 syndrome associated with obesity (Hadaegh, Hasheminia et al. 2013, Pradhan 2014). These
304 studies demonstrate that the rodent models can reflect well some effects of HFD on human
305 metabolic disturbances.

306 Several studies have been conducted in APOE mice on the effects of high fat diets because
307 clinical studies showed *APOE4*-positive individuals have increased risk of metabolic syndrome
308 (Arbones-Mainar, Johnson et al. 2008, Torres-Perez, Ledesma et al. 2016) and obese *APOE4*
309 carriers have increased metabolic disturbances when compared to *APOE3* carriers (Elosua,
310 Demissie et al. 2003). Diverse studies, including ours, showed that there were no differences in
311 baseline glucose levels between *APOE3* and *APOE4* mice on HFD, and that HFD induced worse
312 glucose tolerance in *APOE4* mice than in *APOE3* mice (Table 1). These findings support the
313 observed susceptibility of human *APOE4* carriers to metabolic disturbances, underscoring the
314 importance of diet for *APOE4* individuals in particular.

There are several findings that differ from our work (Table 1). Published studies show that *APOE3* mice on several types of high fat diets gain more weight when compared to *APOE4* mice (Arbones-Mainar, Johnson et al. 2008, Huebbe, Dose et al. 2015, Johnson, Torres et al. 2017), and *APOE3* mice have greater VAT accumulation (Arbones-Mainar, Johnson et al. 2008, Johnson, Torres et al. 2017). In all studies the VAT accumulation reflects the weight gain, with the heavier groups having larger VAT compositions. These differences in VAT accumulation and weight gain across studies could be due to different diet compositions. Our study uses a lard based 45% kcal fat diet; other studies use either a diet where the fat is composed of milk (Arbones-Mainar, Johnson et al. 2008, Huebbe, Dose et al. 2015, Arbones-Mainar, Johnson et al. 2016) or 60% kcal fat from lard (To, Ribe et al. 2011, Johnson, Torres et al. 2017, Johnson, Torres et al. 2019). These findings raise the interesting possibility that both the components and the percentage of fat can differentially affect weight gain in *APOE4* carriers. In humans, healthy *APOE4* carriers have lower Body Mass Index (BMI) (Tejedor, Garcia-Sobreviela et al. 2014) although they remain more susceptible to metabolic and cognitive disturbances.

Studies on the effects of high fat diets on cognition in non-*APOE* mice showed spatial memory deficits and deficits in other cognitive task including novel object recognition and fear condoning in wild type mouse models (Hwang, Wang et al. 2010, Kesby, Kim et al. 2015) and AD mouse models (Barron, Rosario et al. 2013, Knight, Martins et al. 2014, (Knight, Martins et al. 2014, Kesby, Kim et al. 2015, Lin, Hasegawa et al. 2016, Johnson, Torres et al. 2017). Studies on the effects of diet on cognition in *APOE* mice showed either equal levels of impairment in *APOE3* and *APOE4* mice on HFD or increased impairment in *APOE4* mice on HFD depending on the behavioral assay (Johnson, Torres et al. 2017)(Johnson, Torres et al. 2019). We did not observe robust behavioral effects with our behavioral assays. *APOE4* mice exhibited more anxiety like

behavior on the open field but not on the elevated zero. With the Barnes Maze, *APOE4* mice had impairment in spatial learning overall, but diet had only an effect on TD1. Potential effects of diet here may have been obscured by sex differences, which could be addressed in larger cohorts. Previous studies have shown *APOE4* mice have cognitive deficits or decreased neuronal complexity from as early as 3 months and these deficits remain at later ages such as 21 months (Bour, Grootendorst et al. 2008, Rodriguez, Burns et al. 2013, Speidell, Demby et al. 2019), consistent with APOE genotype dependent deficits seen in our study. Performance in cognitive task have differed between sexes also with females performing worse than males (Bour, Grootendorst et al. 2008), further emphasizing the need for these behavioral assays to be replicated with a greater number of animals across sexes.

In humans, obesity has been linked to increased risk of AD, cognitive disturbances, and decreases in structural integrity (Enzinger, Fazekas et al. 2005, Gunstad, Paul et al. 2007). Middle aged obesity is particularly impactful, associated with increased risk of cognitive disturbances and dementia (Whitmer, Gunderson et al. 2005, Gustafson, Karlsson et al. 2007, Tolppanen, Ngandu et al. 2014). However, higher BMI at later ages is protective (Tolppanen, Ngandu et al. 2014), highlighting a complex relationship between BMI and cognition. Interestingly, while obesity in males is associated with increased susceptibility to metabolic disturbances, obesity in females is associated with increased susceptibility to cognitive changes (REF?). Obese females compared to obese males have increased risk of MCI leading to AD, decreased cognitive performances, decreased structural brain integrity (Moser and Pike 2016). *APOE4* females (compared to *APOE4* males) have an equivalent risk of AD, with a significantly earlier age of onset between 65 and 75 years old (Neu, Pa et al. 2017). In mouse studies of *APOE* mice crossed with *5xFAD* (*EFAD*), male obese *E4FAD* mice have higher levels of beta amyloid deposits, glial reactivity, and

inflammatory markers compared to non-obese *E4FAD* mice or obese *E3FAD* mice (Moser and Pike 2017). In female non-obese *EFAD* mice, *APOE4* was associated with higher levels of AD pathology; however the *E3FAD* mice were more affected by HFD, suggesting that the female *E4FAD* mice reached deficits that could not be further exacerbated by diet (Christensen and Pike 2019). Therefore, while our study does not highlight diet specific behavioral deficits data greatly supports the connection between diet and CNS dysfunction.

Chronic systemic inflammation associated with VAT and the alterations in glucose and insulin may be connected to cognitive disturbances (Jones and Rebeck 2018). HFD increases systemic inflammation from the increase in VAT (Hotamisligil, Arner et al. 1995). This increase in inflammation can both induce metabolic disturbances (Xu, Barnes et al. 2003) and increase CNS damage (Kempuraj, Thangavel et al. 2017, Varatharaj and Galea 2017). There is also the possibility that the metabolic disturbances such as glucose intolerance and insulin resistance could more directly lead to CNS damage. Metabolic disturbances have been associated with increased CNS insulin resistance, glucose intolerance (Arnold, Lucki et al. 2014, Kothari, Luo et al. 2017), which can affect brain glucose uptake and neuronal functioning. However, we do not know whether it is the inflammation or metabolic disturbances leading the CNS deficits.

We found that HFD leads to metabolic disturbances particularly in male *APOE4* mice, and in female mice of either *APOE3* or *APOE4* genotypes; however, the underlying mechanisms of this response remain to be defined. Overall, the study implicates *APOE4* positive individuals as more affected by HFD. These connections could affect a large proportion of the population as the increasing rates of obesity increase the risk of metabolic syndrome.

References

Arbones-Mainar, J. M., L. A. Johnson, M. K. Altenburg and N. Maeda (2008). "Differential modulation of diet-induced obesity and adipocyte functionality by human apolipoprotein E3 and E4 in mice." Int J Obes (Lond) **32**(10): 1595-1605.

Arbones-Mainar, J. M., L. A. Johnson, E. Torres-Perez, A. E. Garcia, S. Perez-Diaz, J. Raber and N. Maeda (2016). "Metabolic shifts toward fatty-acid usage and increased thermogenesis are associated with impaired adipogenesis in mice expressing human APOE4." Int J Obes (Lond) **40**(10): 1574-1581.

Arnold, S. E., I. Lucki, B. R. Brookshire, G. C. Carlson, C. A. Browne, H. Kazi, S. Bang, B. R. Choi, Y. Chen, M. F. McMullen and S. F. Kim (2014). "High fat diet produces brain insulin resistance, synaptodendritic abnormalities and altered behavior in mice." Neurobiol Dis **67**: 79-87.

Barron, A. M., E. R. Rosario, R. Elteriefi and C. J. Pike (2013). "Sex-specific effects of high fat diet on indices of metabolic syndrome in 3xTg-AD mice: implications for Alzheimer's disease." PLoS One **8**(10): e78554.

Besser, L. M., D. P. Gill, S. E. Monsell, W. Brenowitz, D. H. Meranus, W. Kukull and D. R. Gustafson (2014). "Body mass index, weight change, and clinical progression in mild cognitive impairment and Alzheimer disease." Alzheimer Dis Assoc Disord **28**(1): 36-43.

Bloor, I. D. and M. E. Symonds (2014). "Sexual dimorphism in white and brown adipose tissue with obesity and inflammation." Horm Behav **66**(1): 95-103.

Bour, A., J. Grootendorst, E. Vogel, C. Kelche, J. C. Dodart, K. Bales, P. H. Moreau, P. M. Sullivan and C. Mathis (2008). "Middle-aged human apoE4 targeted-replacement mice show retention deficits on a wide range of spatial memory tasks." Behav Brain Res **193**(2): 174-182.

Christensen, A. and C. J. Pike (2019). "APOE genotype affects metabolic and Alzheimer-related outcomes induced by Western diet in female EFAD mice." FASEB J **33**(3): 4054-4066.

Elosua, R., S. Demissie, L. A. Cupples, J. B. Meigs, P. W. Wilson, E. J. Schaefer, D. Corella and J. M. Ordovas (2003). "Obesity modulates the association among APOE genotype, insulin, and glucose in men." Obes Res **11**(12): 1502-1508.

Enzinger, C., F. Fazekas, P. M. Matthews, S. Ropele, H. Schmidt, S. Smith and R. Schmidt (2005). "Risk factors for progression of brain atrophy in aging: six-year follow-up of normal subjects." Neurology **64**(10): 1704-1711.

Ghebranious, N., B. Mukesh, P. F. Giampietro, I. Glurich, S. F. Mickel, S. C. Waring and C. A. McCarty (2011). "A pilot study of gene/gene and gene/environment interactions in Alzheimer disease." Clin Med Res **9**(1): 17-25.

Gunstad, J., R. H. Paul, R. A. Cohen, D. F. Tate, M. B. Spitznagel and E. Gordon (2007). "Elevated body mass index is associated with executive dysfunction in otherwise healthy adults." Compr Psychiatry **48**(1): 57-61.

Gustafson, D. R., K. Backman, M. Waern, S. Ostling, X. Guo, P. Zandi, M. M. Mielke, C. Bengtsson and I. Skoog (2009). "Adiposity indicators and dementia over 32 years in Sweden." Neurology **73**(19): 1559-1566.

Gustafson, D. R., C. Karlsson, I. Skoog, L. Rosengren, L. Lissner and K. Blennow (2007). "Mid-life adiposity factors relate to blood-brain barrier integrity in late life." J Intern Med **262**(6): 643-650.

Hadaegh, F., M. Hasheminia, M. Lotfaliany, R. Mohebi, F. Azizi and M. Tohidi (2013). "Incidence of metabolic syndrome over 9 years follow-up; the importance of sex differences in the role of insulin resistance and other risk factors." PLoS One **8**(9): e76304.

Hales, C. M., M. D. Carroll, C. D. Fryar and C. L. Ogden (2017). "Prevalence of Obesity Among Adults and Youth: United States, 2015-2016." NCHS Data Brief(288): 1-8.

Hotamisligil, G. S., P. Arner, J. F. Caro, R. L. Atkinson and B. M. Spiegelman (1995). "Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance." J Clin Invest **95**(5): 2409-2415.

Huang, Y. and R. W. Mahley (2014). "Apolipoprotein E: structure and function in lipid metabolism, neurobiology, and Alzheimer's diseases." Neurobiol Dis **72 Pt A**: 3-12.
Huang, Y., K. H. Weisgraber, L. Mucke and R. W. Mahley (2004). "Apolipoprotein E: diversity of cellular origins, structural and biophysical properties, and effects in Alzheimer's disease." J Mol Neurosci **23**(3): 189-204.

Huebbe, P., J. Dose, A. Schloesser, G. Campbell, C. C. Gluer, Y. Gupta, S. Ibrahim, A. M. Minihane, J. F. Baines, A. Nebel and G. Rimbach (2015). "Apolipoprotein E (APOE) genotype regulates body weight and fatty acid utilization-Studies in gene-targeted replacement mice." Mol Nutr Food Res **59**(2): 334-343.

Hwang, L. L., C. H. Wang, T. L. Li, S. D. Chang, L. C. Lin, C. P. Chen, C. T. Chen, K. C. Liang, I. K. Ho, W. S. Yang and L. C. Chiou (2010). "Sex differences in high-fat diet-induced obesity, metabolic alterations and learning, and synaptic plasticity deficits in mice." Obesity (Silver Spring) **18**(3): 463-469.

Janssen, C. I., D. Jansen, M. P. Mutsaers, P. J. Dederen, B. Geenen, M. T. Mulder and A. J. Kiliaan (2016). "The Effect of a High-Fat Diet on Brain Plasticity, Inflammation and Cognition in Female ApoE4-Knockin and ApoE-Knockout Mice." PLoS One **11**(5): e0155307.

Johnson, L. A., E. R. Torres, S. Impey, J. F. Stevens and J. Raber (2017). "Apolipoprotein E4 and Insulin Resistance Interact to Impair Cognition and Alter the Epigenome and Metabolome." Sci Rep **7**: 43701.

Johnson, L. A., E. R. Torres, S. Weber Boutros, E. Patel, T. Akinyeke, N. J. Alkayed and J. Raber (2019). "Apolipoprotein E4 mediates insulin resistance-associated cerebrovascular dysfunction and the post-prandial response." J Cereb Blood Flow Metab **39**(5): 770-781.

Jones, N. S. and G. W. Rebeck (2018). "The Synergistic Effects of APOE Genotype and Obesity on Alzheimer's Disease Risk." Int J Mol Sci **20**(1).

Kempuraj, D., R. Thangavel, G. P. Selvakumar, S. Zaheer, M. E. Ahmed, S. P. Raikwar, H. Zahoor, D. Saeed, P. A. Natteru, S. Iyer and A. Zaheer (2017). "Brain and Peripheral Atypical Inflammatory Mediators Potentiate Neuroinflammation and Neurodegeneration." Front Cell Neurosci **11**: 216.

Kesby, J. P., J. J. Kim, M. Scadeng, G. Woods, D. M. Kado, J. M. Olefsky, D. V. Jeste, C. L. Achim and S. Semenova (2015). "Spatial Cognition in Adult and Aged Mice Exposed to High-Fat Diet." PLoS One **10**(10): e0140034.

Knight, E. M., I. V. Martins, S. Gumusgoz, S. M. Allan and C. B. Lawrence (2014). "High-fat diet-induced memory impairment in triple-transgenic Alzheimer's disease (3xTgAD) mice is independent of changes in amyloid and tau pathology." Neurobiol Aging **35**(8): 1821-1832.

Kothari, V., Y. Luo, T. Tornabene, A. M. O'Neill, M. W. Greene, T. Geetha and J. R. Babu (2017). "High fat diet induces brain insulin resistance and cognitive impairment in mice." Biochim Biophys Acta Mol Basis Dis **1863**(2): 499-508.

Lin, B., Y. Hasegawa, K. Takane, N. Koibuchi, C. Cao and S. Kim-Mitsuyama (2016). "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 **5**(6).

Liu, C. C., C. C. Liu, T. Kanekiyo, H. Xu and G. Bu (2013). "Apolipoprotein E and Alzheimer disease: risk, mechanisms and therapy." Nat Rev Neurol **9**(2): 106-118.

Macotela, Y., J. Boucher, T. T. Tran and C. R. Kahn (2009). "Sex and depot differences in adipocyte insulin sensitivity and glucose metabolism." Diabetes **58**(4): 803-812.

Mathieu, P., P. Poirier, P. Pibarot, I. Lemieux and J. P. Despres (2009). "Visceral obesity: the link among inflammation, hypertension, and cardiovascular disease." Hypertension **53**(4): 577-584.

Medrikova, D., Z. M. Jilkova, K. Bardova, P. Janovska, M. Rossmeisl and J. Kopecky (2012). "Sex differences during the course of diet-induced obesity in mice: adipose tissue expandability and glycemic control." Int J Obes (Lond) **36**(2): 262-272.

Moser, V. A. and C. J. Pike (2016). "Obesity and sex interact in the regulation of Alzheimer's disease." Neurosci Biobehav Rev **67**: 102-118.

Moser, V. A. and C. J. Pike (2017). "Obesity Accelerates Alzheimer-Related Pathology in APOE4 but not APOE3 Mice." eNeuro **4**(3).

Neth, B. J. and S. Craft (2017). "Insulin Resistance and Alzheimer's Disease: Bioenergetic Linkages." Front Aging Neurosci **9**: 345.

Neu, S. C., J. Pa, W. Kukull, D. Beekly, A. Kuzma, P. Gangadharan, L. S. Wang, K. Romero, S. P. Arneric, A. Redolfi, D. Orlandi, G. B. Frisoni, R. Au, S. Devine, S. Auerbach, A. Espinosa, M. Boada, A. Ruiz, S. C. Johnson, R. Koscik, J. J. Wang, W. C. Hsu, Y. L. Chen and A. W. Toga (2017). "Apolipoprotein E Genotype and Sex Risk Factors for Alzheimer Disease: A Meta-analysis." JAMA Neurol **74**(10): 1178-1189.

Pradhan, A. D. (2014). "Sex differences in the metabolic syndrome: implications for cardiovascular health in women." Clin Chem **60**(1): 44-52.

Profenno, L. A., A. P. Porsteinsson and S. V. Faraone (2010). "Meta-analysis of Alzheimer's disease risk with obesity, diabetes, and related disorders." Biol Psychiatry **67**(6): 505-512.

Raber, J., Y. Huang and J. W. Ashford (2004). "ApoE genotype accounts for the vast majority of AD risk and AD pathology." Neurobiol Aging **25**(5): 641-650.

Rodriguez, G. A., M. P. Burns, E. J. Weeber and G. W. Rebeck (2013). "Young APOE4 targeted replacement mice exhibit poor spatial learning and memory, with reduced dendritic spine density in the medial entorhinal cortex." Learn Mem **20**(5): 256-266.
Schulz, T. J. and Y. H. Tseng (2013). "Brown adipose tissue: development, metabolism and beyond." Biochem J **453**(2): 167-178.

Seibenhener, M. L. and M. C. Wooten (2015). "Use of the Open Field Maze to measure locomotor and anxiety-like behavior in mice." J Vis Exp(96): e52434.

Speidell, A. P., T. Demby, Y. Lee, O. Rodriguez, C. Albanese, J. Mandelblatt and G. W. Rebeck (2019). "Development of a Human APOE Knock-in Mouse Model for Study of Cognitive Function After Cancer Chemotherapy." Neurotox Res **35**(2): 291-303.

Tejedor, M. T., M. P. Garcia-Sobreviela, M. Ledesma and J. M. Arbones-Mainar (2014). "The apolipoprotein E polymorphism rs7412 associates with body fatness independently of plasma lipids in middle aged men." PLoS One **9**(9): e108605.

To, A. W., E. M. Ribe, T. T. Chuang, J. E. Schroeder and S. Lovestone (2011). "The epsilon3 and epsilon4 alleles of human APOE differentially affect tau phosphorylation in hyperinsulinemic and pioglitazone treated mice." PLoS One **6**(2): e16991.

Tolppanen, A. M., T. Ngandu, I. Kareholt, T. Laatikainen, M. Rusanen, H. Soininen and M. Kivipelto (2014). "Midlife and late-life body mass index and late-life dementia: results from a prospective population-based cohort." J Alzheimers Dis **38**(1): 201-209.

562 Torres-Perez, E., M. Ledesma, M. P. Garcia-Sobreviela, M. Leon-Latre and J. M. Arbones-Mainar
563 (2016). "Apolipoprotein E4 association with metabolic syndrome depends on body fatness."
564 Atherosclerosis **245**: 35-42.
565
566 Varatharaj, A. and I. Galea (2017). "The blood-brain barrier in systemic inflammation." Brain
567 Behav Immun **60**: 1-12.
568
569 Whitmer, R. A., E. P. Gunderson, E. Barrett-Connor, C. P. Quesenberry, Jr. and K. Yaffe (2005).
570 "Obesity in middle age and future risk of dementia: a 27 year longitudinal population based study."
571 BMJ **330**(7504): 1360.
572
573 Whitmer, R. A., D. R. Gustafson, E. Barrett-Connor, M. N. Haan, E. P. Gunderson and K. Yaffe
574 (2008). "Central obesity and increased risk of dementia more than three decades later." Neurology
575 **71**(14): 1057-1064.
576
577 Xu, H., G. T. Barnes, Q. Yang, G. Tan, D. Yang, C. J. Chou, J. Sole, A. Nichols, J. S. Ross, L. A.
578 Tartaglia and H. Chen (2003). "Chronic inflammation in fat plays a crucial role in the development
579 of obesity-related insulin resistance." J Clin Invest **112**(12): 1821-1830.
580
581 Zade, D., A. Beiser, R. McGlinchey, R. Au, S. Seshadri, C. Palumbo, P. A. Wolf, C. DeCarli and
582 W. Milberg (2013). "Apolipoprotein epsilon 4 allele modifies waist-to-hip ratio effects on
583 cognition and brain structure." J Stroke Cerebrovasc Dis **22**(2): 119-125.
584
585

Figure Titles and Legends

Figure 1: HFD increases weight gain and glucose intolerance. Weight gain comparison from pre diet to week 12 on diet across *APOE* genotypes and sex. Initial weights of all mice in grams (A). Diet associated weight gain of female *APOE3* and *APOE4* mice (B) and male *APOE3* and *APOE4* mice (C). Direct comparison of male and female weight gain at week 11 on diet (D). E3 LFD: light blue, *APOE3* mice on a LFD, E3 HFD: dark blue, *APOE3* mice on a HFD, E4 LFD: light red, *APOE4* mice on a LFD, E4 HFD: dark red, *APOE4* mice on a HFD.

Three-way ANOVA Tukey Test Multiple Comparison, N=5-9. A) ****p<0.0001. B) *p<0.04. **p<0.003. C) ****p<0.0001. D) **p<0.0046.

Figure 2: HFD increases baseline glucose and glucose intolerance. Comparison of baseline glucose levels in male and female *APOE3* and *APOE4* mice, first comparing within sex differences then across sex differences (A). Correlational analyses between weight, glucose levels, and sex. Lines indicate significant correlations (B). Glucose tolerance testing in female (C) and male (D) *APOE3* and *APOE4* mice. Area Under the Curve (AUC)- complete deviation from baseline glucose levels, comparing within sex differences and across sex differences (E). Correlation between genotype, weight gain, sex and glucose levels at 30 minutes. Lines indicate significant correlations (F).

A,C-E) N=4-6. Three Way ANOVA Tukey Test Multiple Comparison. A) **p<0.002, ****p<0.0001. C) **p<0.003 all groups deviate from baseline at 15 minutes, ****p<0.0001 HFD and LFD *APOE4* deviates from baseline at 30 and 60 minutes. D) **p<0.002 all groups deviate

609 from baseline at 15 minutes, *p<0.02 all groups deviate from baseline at 30 minutes, ***p<0.0002
610 HFD *APOE4* deviates from baseline at 60 minutes. E) *p<0.02, **p<0.01, ***p<0.0003,
611 ****p<0.0001. B&F) Linear Regression. B) *APOE3* males (N=12): R^2=0.68, p=0.001. APOE4
612 males (N=11): R^2=0.63, p=0.004. F) *APOE3* males (N=12): R^2=0.75, p=0.0002. *APOE3* females
613 (N=9): R^2=0.72, p=0.004. *APOE4* females (N=12): R^2=0.42, p=0.02.
614
615 **Figure 3:** HFD increases VAT in *APOE3* and *APOE4* mice. Second panels in A-D show the same
616 data with a different analysis. Representative image of VAT and SAT in LFD mouse and HFD
617 mouse. S=subcutaneous adipose tissue, V= visceral adipose tissue, K=kidneys (A). Within sex and
618 across sex quantification of VAT in *APOE3* and *APOE4* mice (B). Correlation of glucose
619 intolerance and VAT accumulation across *APOE* genotypes and across sex. Lines indicate
620 significant correlations (C). Correlation of weight gain and VAT accumulation across *APOE*
621 genotypes and across sex. Lines indicate significant correlations (D).
622
623 B) N=5-9. Within sex comparison: ****p<0.0001, Three-way ANOVA Tukey's multiple
624 comparison. Across sex comparison: *p<0.02, Three-way ANOVA Tukey's multiple comparison.
625 C-D) Linear Regression analyses. C) *APOE3* female (N=6): R^2=0.52, p=0.005; *APOE4* male
626 (N=7): R^2=0.48, p=0.01; *APOE4* female (N=14): R^2=0.43, p=0.003. D) *APOE4* male (N=14):
627 R^2=0.62, p=0.03.
628
629 **Figure 4:** HFD increases SAT in *APOE3* and *APOE4* mice. Within sex and across sex
630 quantification of VAT in *APOE3* and *APOE4* mice (A). Correlation of VAT and SAT
631 accumulation(C).

A) N=5-9, p<0.04, Three-way ANOVA Tukey's multiple comparison. B) N=59, Linear Regression R^2=0.47, p=<0.0001

Figure 5: Male *APOE* mice have decreased BAT. Representative image of BAT in the neck in a LFD mouse and HFD mouse (A). Comparison of BAT intensity in male and female *APOE3* and *APOE4* mice (B). Correlation of genotype and weight to BAT intensity then sex and weight to BAT intensity Lines indicate significant correlations (C). B=brown adipose tissue, W= white adipose tissue, S=spine, TAT-total adipose tissue

B) N=5-9, **p<.004, Three Way ANOVA Tukey Test Multiple Comparison. C) Linear Regression. *APOE3* male (N=17): R^2=0.25, p=0.04; *APOE3* female (N=15): R^2=0.32, p=0.03; *APOE4* male (N=14): R^2=0.42, p=0.01.

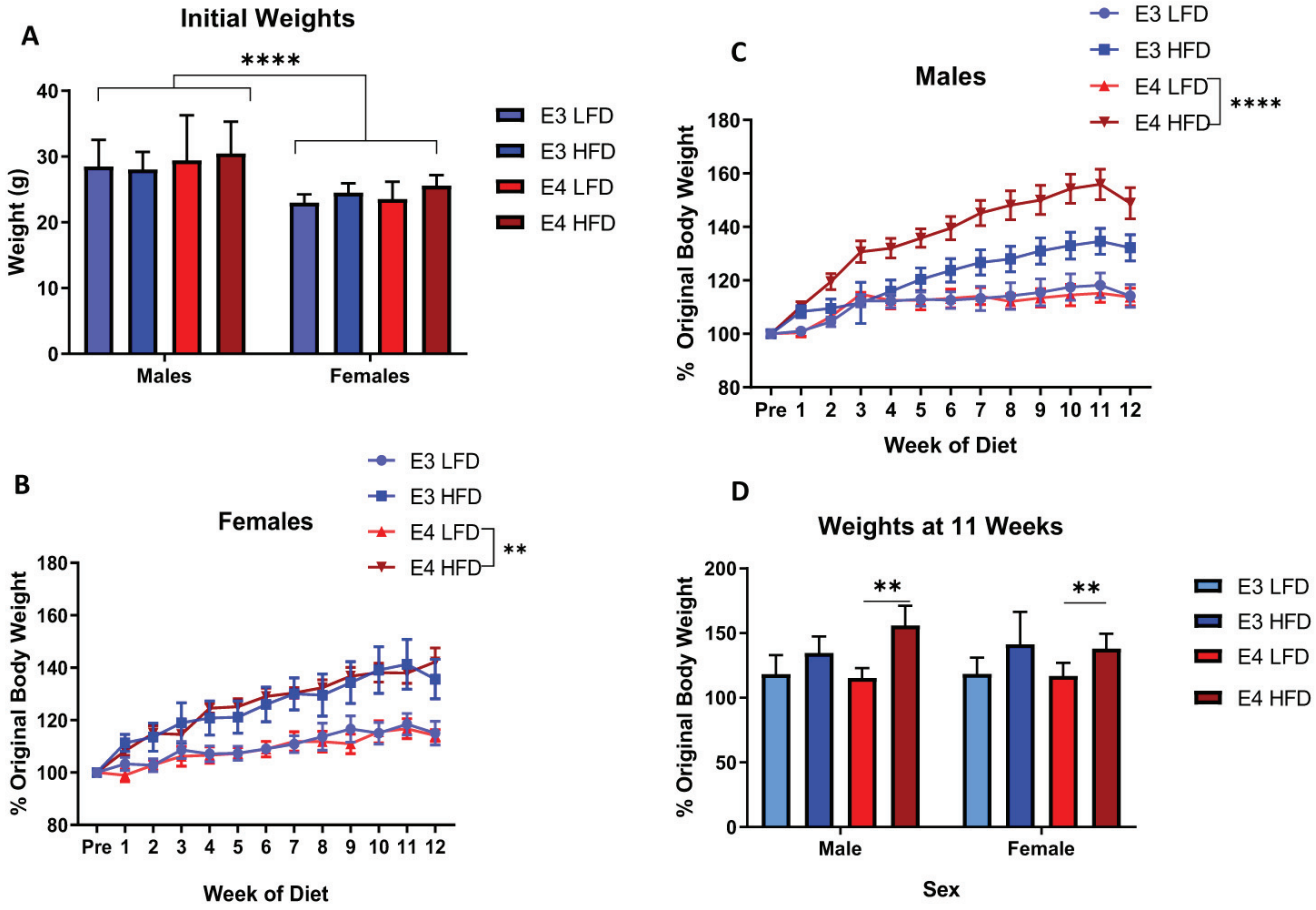
Figure 6: Effects of diet on locomotor activity and anxiety-like behavior. Mice were placed on an open field apparatus and locomotion was recorded. Average speed on open field test analyzed by *APOE* genotype and diet (A). Mice were placed on the open field apparatus and elevated zero maze and anxiety-like behavior were analyzed. Time spent in the Center Zone of the Open Field Test (B). Time spent in the open arms of the Elevated Zero maze (C).

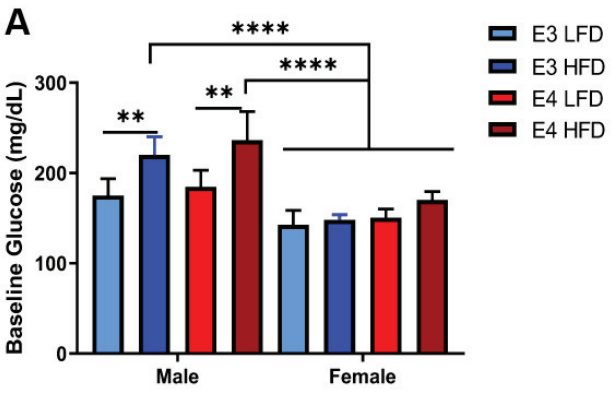
A-C) N=13-15 mice. Two-Way ANOVA Sidak's multiple comparison test. B) *p<0.05, **p<0.002.

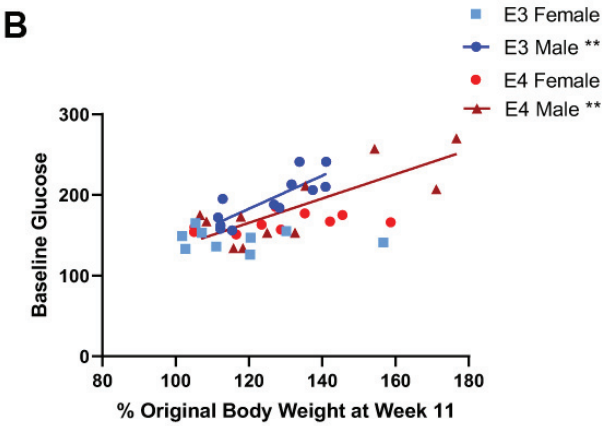
Figure 7: Effects of diet on Barnes Maze performance. Mice were trained on the Barnes Maze for four consecutive days and memory acquisition measured. Latency to first nose poke of the escape hole (A). Latency to escape from the Barnes Maze (B).

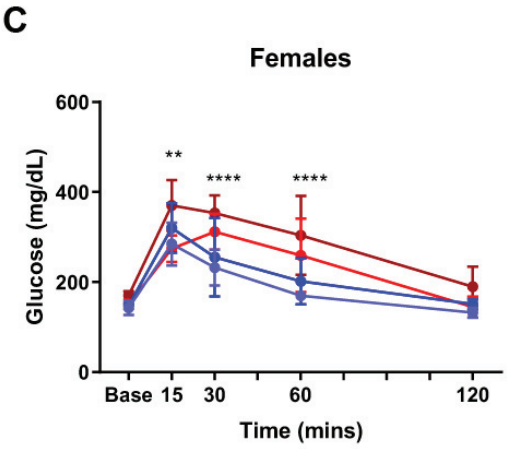
A-B) N=13-15. *P<.03, $P<.05, **P<.004, Two-Way ANOVA, Sidak's Multiple Comparison.

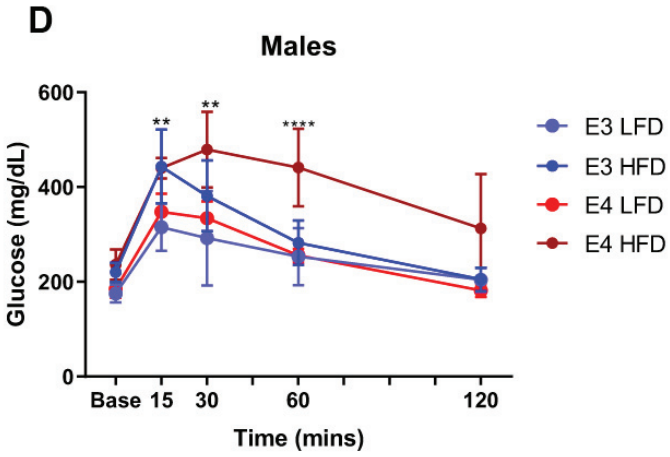
Table 1: Studies of the effects of a HFD on *APOE3* and *APOE4* mice.

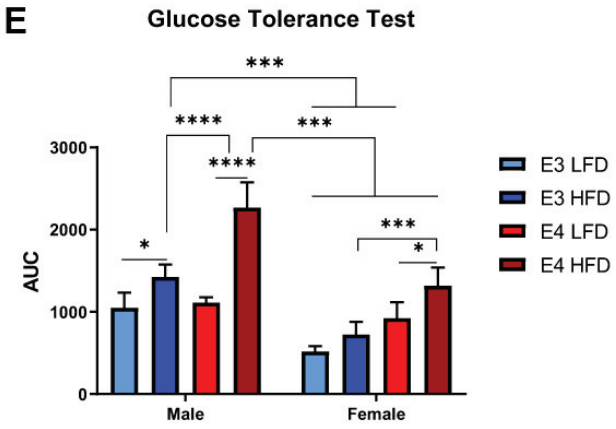


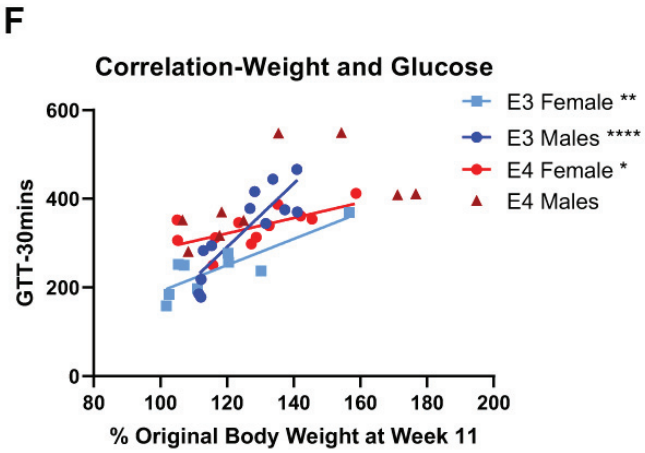


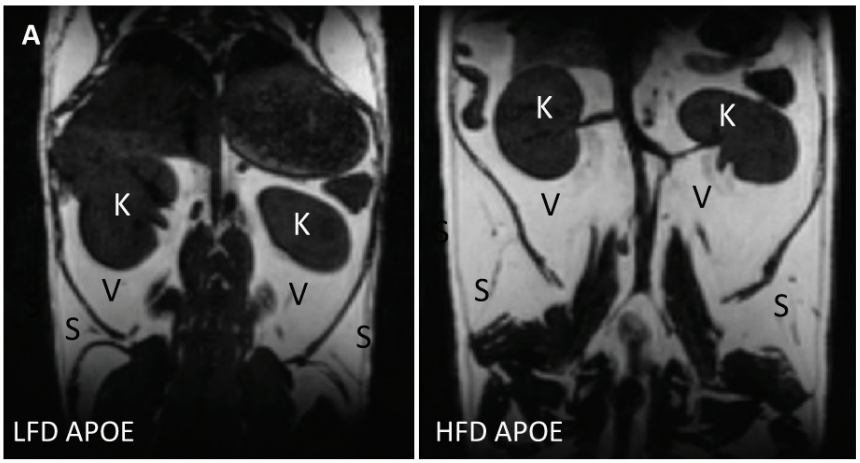


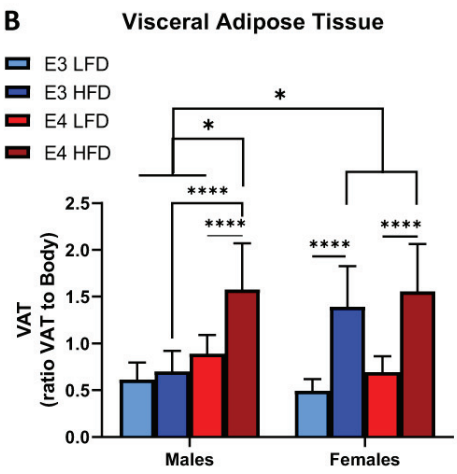


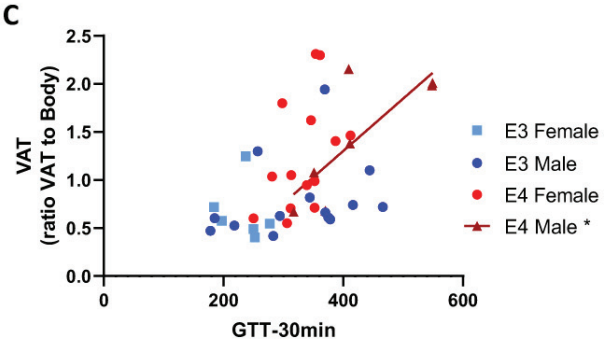


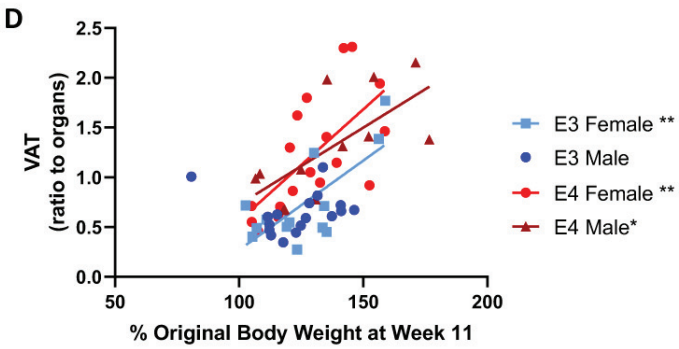


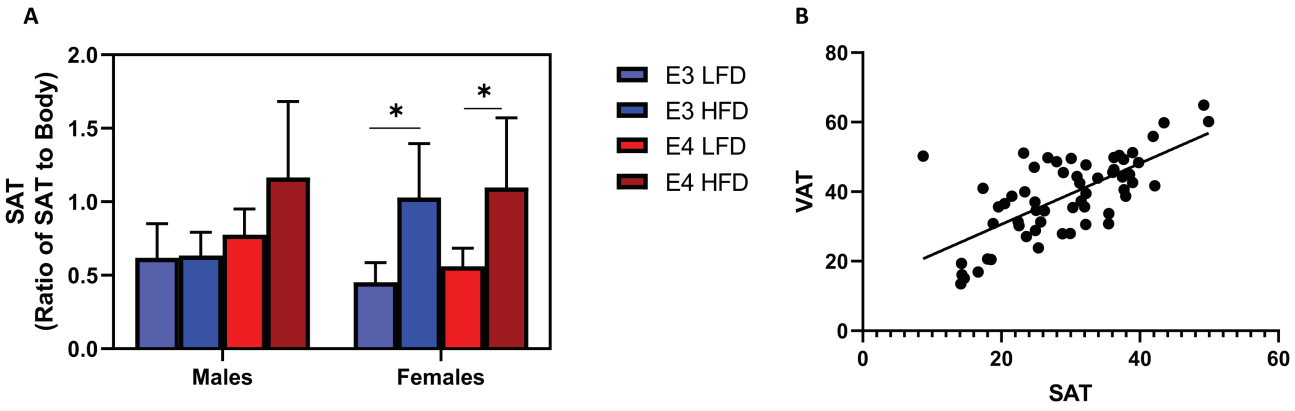


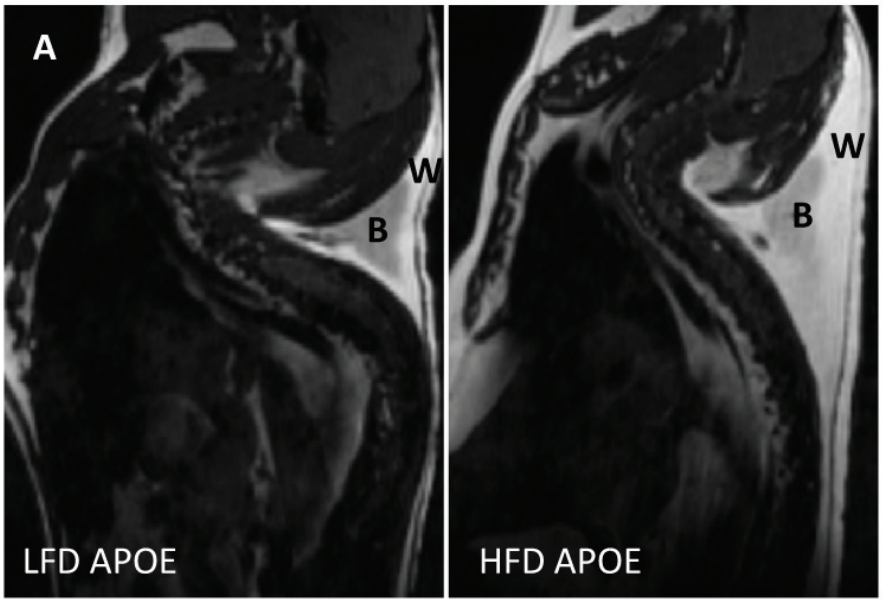


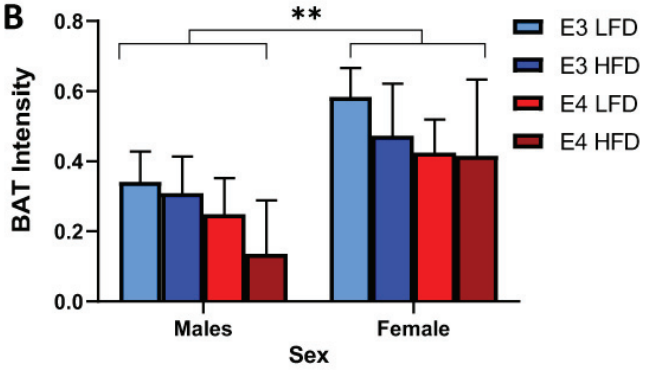


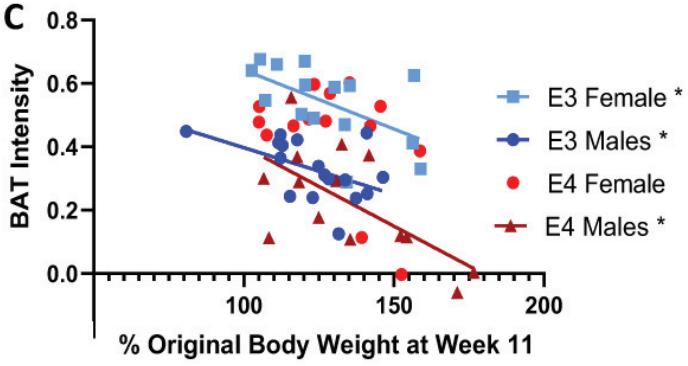


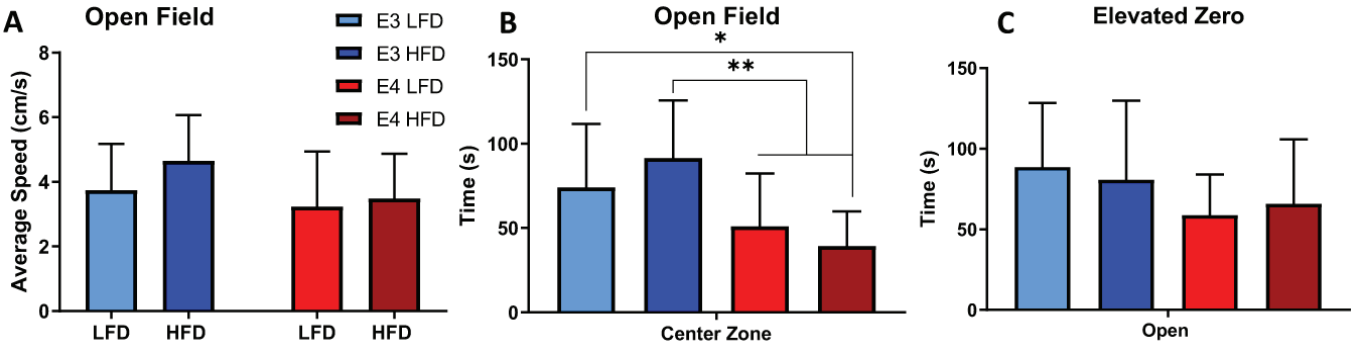


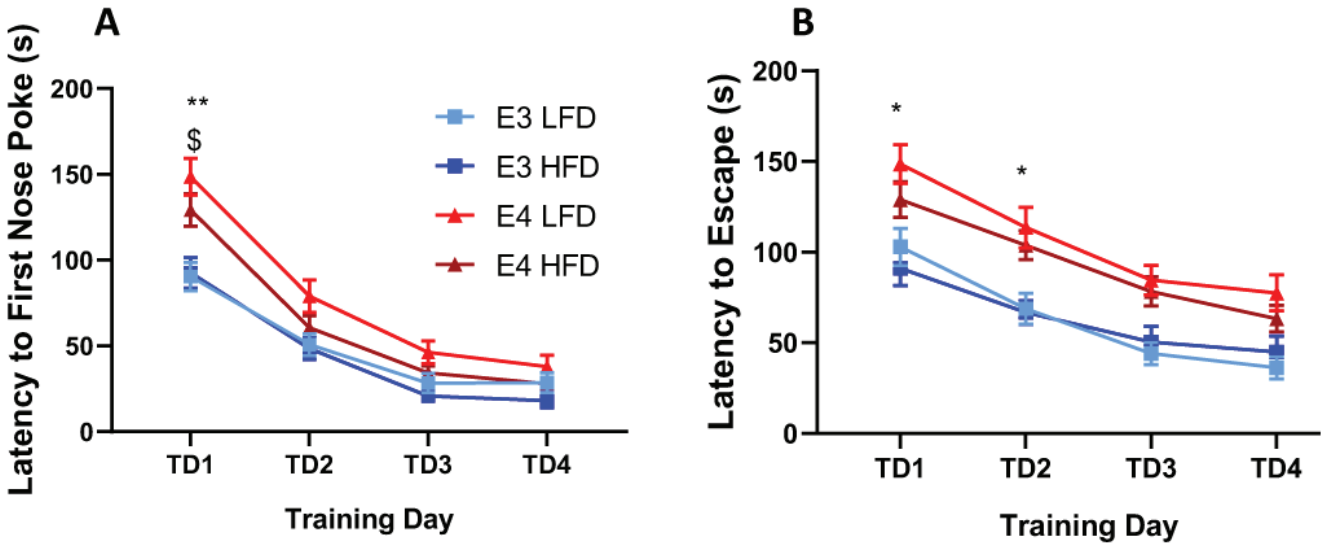












Reference	Onset/ Duration	Sex	Dietary Composition	Metabolic Findings	Cognitive Findings
Arbones-Mainar et al., 2008	2 months/ 8 weeks	Male	21% (w/w) fat and 0.2% (w/w) cholesterol	• Weight gain: E3 HFD>E4 HFD • SAT accumulation: E3 HFD=E4 HFD • VAT accumulation: E3 HFD>E4 HFD • Baseline Glucose: E3 HFD=E4 HFD • Glucose intolerance: E4 HFD> E3 HFD	N/A
To et al., 2011	3 months/ 32 weeks	Male	60% kcal fat from lard	• Baseline Glucose: E3 HFD= E4 HFD • Glucose intolerance: E3 HFD=E4 HFD	N/A
Huebbe et al., 2014	6-8 weeks/ 8 months	Female	21% fat from milk	• Weight gain: E3 HFD>E4 HFD	N/A
Arbones-Mainar et al., 2016	2 months/ 1,2,6,10 months	Male	21% (w/w) fat from milk and 0.2% (w/w) cholesterol	• Weight gain: E3 HFD> E4 HFD • Baseline Glucose: E3 HFD= E4 HFD	N/A
Johnson et al., 2017	9 months/ 6 months	Female	60% kcal fat from lard	• Weight gain: E3 HFD>E4 HFD • VAT accumulation: E3 HFD>E4 HFD • Baseline Glucose: E3 HFD=E4 HFD • Glucose intolerance: E4 HFD>E3 HFD	• Object Recognition Impairment: E3 HFD=E4 HFD • Cued Fear Memory: E3 HFD=E4 HFD • Spatial Memory: E4 HFD> E3 HFD
Johnson et al., 2019	9 months/ 6 months	Female	60% kcal fat from lard	• Weight gain: E3 HFD> E4 HFD • SAT accumulation: E3 HFD= E4 HFD • VAT accumulation: E3 HFD> E4 HFD • Baseline Glucose: E3 HFD=E4 HFD • Glucose intolerance: E3 HFD=E4 HFD	• Morris Water Maze: E3 HFD=E4 HFD