Effects of a high-fat diet in health and in Alzheimer's disease: a gender comparison study

A thesis submitted to the University of Manchester for the degree of Doctor of Philosophy in the Faculty of Life Sciences

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Abstract of thesis

Submitted by Isaura Martins for the degree of Doctor of Philosophy and entitled:

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The prevalence of obesity is growing worldwide partly due to an increase in consumption of diets high in fat. Obesity is known as a risk factor for developing Alzheimer's disease (AD) later in life. Both obesity and AD are associated with cognitive deficits and experimental high-fat diets can impair memory in cognitively normal rodents but also worsen memory deficits in AD mouse models. What is still unclear is the molecular mechanisms behind the detrimental effects of a high-fat diet on memory and if sex can influence its effect.

Data in this thesis demonstrated that compared to females, male control non-transgenic (Non-Tg) mice had earlier deficits in memory after a high-fat diet that were associated with hyperinsulinemia. However, female Non-Tg mice were more vulnerable to ultrastructural changes in mitochondria morphology and loss of synapses after 6 months of a high-fat diet, changes that were similar to those observed in control-fed female triple-transgenic mice (3xTgAD). Finally, the memory deficits observed after a high-fat diet in cognitively normal mice were not associated with obesity and adiposity, as treatment with resveratrol (RSV) an anti-obesogenic compound, attenuated body weight gain and adipose tissue but failed to reverse memory impairment. In control fed 3xTgAD mice, RSV rescued memory deficits. In all experiments a high-fat diet had no detectable effect on cognitive impairment in 3xTgAD mice.

In conclusion, the present thesis demonstrates that the sex-dependent differences in the effect a high-fat diet on memory are likely due to hyperinsulinemia and mitochondrial impairment and do not depend on obesity phenotype. These results demonstrate the importance of gender when studying both obesity and AD and are relevant for future clinical trials.

Declaration

I declare that no portion of the work referred to in this thesis has been submitted in support of an application for another degree or qualification of this or any other university or institute of learning.

Isaura Martins, 28th March 2015

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Alternative format for submission

This thesis has been submitted in the alternative format. The format consists of a general introduction; four experimental chapters, each representing a paper submitted or to be submitted for publication; a general discussion; an appendix and references. This format has been approved by the Faculty of Life Sciences for submission (presentation of theses policy, section 7).

Publications

Elysse M. Knight, Isaura V.A. Martins, Sarah Gumusgoz, Stuart M. Allan, Catherine 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." Neurobiology of Aging 35(8): 1821-1832.

Authorship details

The experimental Chapters 2 and 5 contain some experimental work that was performed by others. Details of the contribution of each author to the work are presented below.

Chapter 2

Authors: Elysse M. Knight, Isaura Martins, Sarah Gumusgoz, Stuart M. Allan, Catherine B. Lawrence.

This chapter contains work from Elysse M. Knight thesis, but only work performed by Isaura Martins is discussed in the present thesis. Elysse M. Knight performed all the behavioural experiments, assessed body weight and epididymal fat weight and all respective analyses. Isaura Martins performed Aβ oligomer enzyme-linked immunosorbent assay and microglia immunohistochemistry and respective analyses. Sarah Gumusgoz performed immunohistochemistry for Aβ plaques and tau and their respective analyses. Elysse M. Knight and Catherine B. Lawrence conceived and designed the experiments. Catherine B. Lawrence and Stuart M. Allan provided critical review.

Chapter 3 and 4

Authors: Isaura Martins, Stuart M. Allan, Catherine B. Lawrence.

All experimental work was performed by Isaura Martins. Isaura Martins and Catherine B. Lawrence conceived and designed the experiments. Isaura Martins wrote the paper, and Catherine B. Lawrence and Stuart M. Allan provided critical review.

Chapter 5

Authors: Isaura Martins, Helen Parker, Stuart M. Allan, Catherine B. Lawrence.

Helen Parker performed the resveratrol dosage, body weight and water intake assessment of mice until 4 months of age. All remaining experimental work was performed by Isaura Martins. Isaura Martins and Catherine B. Lawrence conceived and designed the experiments. Isaura Martins wrote the paper, and Catherine B. Lawrence and Stuart M. Allan provided critical review.

List of abbreviations

3xTgAD	Triple transgenic mouse model of Alzheimer's disease
A	Almond
AD	Alzheimer's disease
AICD	APP intracellular domain
ANOVA	Analysis of variance
APH-1	Anterior pharynx defective 1
ApoE	Apolipoprotein E
APOE	Apolipoprotein E gene
APOE4	Apolipoprotein E gene allele 4
APP	Amyloid precursor protein
APPsα	Larger soluble extracellular N-terminal portions of APP
LTD	Long-term depression
APPsβ	Shorter soluble N-terminus of APP
ATP	Adenosine triphosphate
Aβ	Amyloid-beta
BACE1	β-site APP-cleaving enzyme 1
BAT	Brown adipose tissue
BBB	Blood-brain barrier
BCA	Bicinchoninic acid assay
BDNF	Brain-derived neurotrophic factor
BMI	Body mass index
bpm	Beats per minute
C83	C-terminal of 83 residues
C99	Amyloidogenic C-terminal fragment
Ca^{2+}	Calcium
CAA	Cerebral amyloid angiopathy

CBF	Cerebral blood flow
CNS	Central nervous system
CR	Caloric restriction
CRP	C-reactive protein
CSF	Cerebrospinal fluid
Ctrl	Control
Drp1	Dynamin-related protein 1
ECL	Enhanced chemiluminescence
ELISA	Enzyme-linked immunosorbent assay
ER	Endoplasmatic reticulum
ETC	Electron transport chain
FAD	Familial Alzheimer's disease
FFAs	Free fatty acids
FTD	Frontotemporal dementia
g	grams
h	Hour
HF	High-fat
HRP	Horseradish peroxidase
ICAM-1	Intercellular adhesion molecule 1
IDE	Insulin-degrading enzyme
IL-6	Interlukin-6
IRS	Insulin receptor substract
JAK	Janus kinase
Kg	Kilograms
LPS	Lipopolysaccharide
LRP	Low-density receptor-related protein
LTD	Long-term depression
LTP	Long-term potentiation

mg	milligrams
min	Minutes
mtDNA	Mitochondrial DNA
MWM	Morris water maze
NAD	Nicotinamide adenine dinucleotide
NAFLD	Non-alcoholic fatty liver disease
NEP	Neprilysin
NFT	Neurofibrillary tangles
NFκB	Pro-inflammatory transcription factor nuclear factor κB
Non-Tg	Non-transgenic
NOR	Novel object recognition test
NVU	Neurovascular unit
PBST	Phosphate buffered saline with tween 20
PEN-1	Presenilin enhancer 1
PI3K	Phosphotidylionositol-3 kinase
PKC	Protein kinase C
PS	Presenilin
PS1	Presenilin 1
PS2	Presenilin 2
PSD-95	Postsynaptic density protein 95
RAGE	Receptor for advanced glycation end products
ROS	Reactive species of oxygen
RSV	Resveratrol
RT	Room temperature
SAD	Sporadic Alzheimer’s disease
SDS	Sodium dodecyl sulfate
SEM	Standard error of the mean
SFAs	Saturated fatty acids

SIRT1	nicotinamide adenine dinucleotide-dependent protein deacetylase sirtuin 1
SOCS3	Suppressor of cytokine signalling 3
SR	Smell recognition test
STAT	Signal transducer and activator of transcription
STAT3	Signal transducer and activator of transcription 3
T2D	Diabetes type 2
TEM	Transmission electron microscopy
Tg	Transgenic
TJs	Tight junctions
TLR4	Toll like receptor 4
TNF-α	Tumor necrosis factor alpha
UCP-1	Uncoupling protein-1
V	Vanilla
VCAM-1	Vascular cell adhesion molecule 1
VDAC1	Voltage-dependent anion channel 1
WAT	White adipose tissue
WHO	World Health Organization

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First of all I would like to thank my supervisor Catherine Lawrence for all the patience, support and knowledge throughout these 4 years. For being always positive, for trusted me and for giving me the opportunity to always follow my gut feeling and my ideas, for teaching me how to think and look outside the box. Thank you as well for the talks and for welcoming me since the first day in Manchester. I really appreciate all that and it has been an amazing journey.

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Chapter 1

Introduction

1.1. Overview

Consumption of western diets that are rich in fatty acids and cholesterol are increasing worldwide and contributing to an increase in the obese population. Obesity, which is defined by an increase in adipose tissue, has been associated with effects on cognitive function including impairments in memory (Waldstein and Katzel, 2005, Bruce-Keller et al., 2009, Kanoski and Davidson, 2011). Alzheimer's disease (AD), the major cause of dementia, is also characterised by a progressive memory decline and it is known that midlife obesity is associated with increased risk of developing AD later in life (Tolppanen et al., 2014).

AD prevalence is affected by age, gender and other risk factors, such as obesity, however it is known that more women than men develop AD (Ott et al., 1998, Sinforiani et al., 2010, Viña and Lloret, 2010), showing a clear effect of gender. In obesity, the majority of experimental studies showing negative effects of a high-fat diets on memory are in male rodents although it is known that the metabolic response to a high-fat diet can differ between males and females, since in general women have more body fat and are more susceptible to weight gain than men (Power and Schulkin, 2008).

Although it is known that both AD and obesity share common features, such as increased oxidative stress, blood-brain barrier (BBB) impairment and inflammation, the real mechanisms underlying the detrimental effect of high-fat diet on memory and its association with AD is still not clear. Moreover, neither is the effect of gender on diet-induced memory impairment.

The aim of this thesis is to understand the effect of a high-fat diet on memory in both cognitively normal and cognitively impaired mice (AD model) and if gender can influence cognitive outcome. With this it will be possible to better understand the molecular mechanism associated with memory deficits in both obesity and AD.

1.2. Alzheimer's disease

AD is the most common form of dementia in the elderly and accounts for 50-60% of all cases (Blennow et al., 2006). Age is the main risk factor for the development of the disease, and

population-based studies in Europe suggest that the age-specific prevalence for AD in people aged above 65 years old is 4.4 %, which almost doubles every 5 years after the age of 65 (Qiu et al., 2009). 75% of individuals with AD have no family history of the disease and it is associated with the sporadic AD (SAD), whilst approximately 25% of AD cases are familiar (FAD). About 1-6% of AD cases is early onset (≤ 65 years old) and about 60% of these are familial (Bird, 2014).

AD is a chronic and neurodegenerative disorder that normally begins with a subtle failure of episodic memory that slowly becomes more severe. It is characterised by a progressive and irreversible memory decline due to loss of synapses and neurones in brain areas such as the hippocampus, frontal cortex, basal forebrain, entorhinal and temporal cortices (Jacobsen et al., 2005). Deficits in formation and retention of new episodic memories are the main typical symptoms (Sperling et al., 2010), however other symptoms include language breakdown, depression, apathy, agitation, aggression and psychosis (Nakaaki et al., 2008, Querfurth and LaFerla, 2010, Usman et al., 2010, Benoit et al., 2012, Bidzan et al., 2012, Grossi et al., 2013, Van der Mussele et al., 2013, Kwak et al., 2014). As the disease progresses the dependency of AD patients by their caregivers increases, and although there are limited symptomatic treatments for AD, no cure has been found to date.

1.2.1 Alzheimer's disease pathology and mechanisms

AD is characterized pathologically by two hallmarks: neuritic/senile plaques, composed by amyloid-beta (Aβ) deposits, and intracellular neurofibrillary tangles (NFT), composed of filamentous aggregates of hyperphosphorylated tau protein (Selkoe, 2001). NFTs derive from abnormal hyperphosphorylation of the intracellular protein tau, a microtubule-associated protein responsible for microtubule assembly and protein trafficking, maintenance of neuronal structure, axonal transport, and neuronal plasticity (Lindwall and Cole, 1984). Plaques aggregate in extracellular spaces and within the cerebral vasculature, and are mainly composed by insoluble Aβ deposits that result from the cleavage of amyloid precursor protein (APP) (Figure 1). Besides Aβ and tau, reactive microglia cells and activated astrocytes are also observed within the AD brain (Blennow et al.,

2006), which suggest a prominent activation of inflammatory processes and a response of the innate immune system against the disease (Citron, 2010). Moreover, plenty of evidence suggest that Aβ and tau do not act alone, and inflammation (Okello et al., 2009a, Okello et al., 2009b, Perry et al., 2010, Mattsson et al., 2011, Perry and Teeling, 2013, Perry and Holmes, 2014), oxidative damage and mitochondrial impairment (Maurer et al., 2000, Hirai et al., 2001, Reddy et al., 2004, Caspersen et al., 2005, Wang et al., 2005, Devi et al., 2006, Manczak et al., 2006, Reddy and Beal, 2008) are key factors in AD.

1.2.1.1 APP processing

APP is a type-1 transmembrane protein that contains Aβ at the C-terminal (Hardy and Selkoe, 2002, Carlson, 2003, LaFerla and Oddo, 2005, De-Paula et al., 2012). In addition to being mainly present in the cell membrane of neurones, APP is also found in various other organelles, such as the endoplasmic reticulum (ER), Golgi apparatus, and mitochondria (Rhein and Eckert, 2007). APP is metabolized by two different pathways, resulting in the formation or the prevention of Aβ: amyloidogenic or non-amyloidogenic pathways respectively. The non-amyloidogenic pathway involves membrane-associated metalloprotease α-secretase that cleaves APP between the residues 16 and 17 of the Aβ domain, which prevents the formation of toxic forms of Aβ. As a result, large soluble extracellular N-terminal portions of APP (APPsα) and C-terminal fragment of 83 residues (C83) are produced (LaFerla and Oddo, 2005, Blennow et al., 2006) (Figure 1).

In the amyloidogenic pathway, APP is cleaved by β- and γ-secretases. Firstly APP is cleaved by β-secretase at the N-terminus region of Aβ, leading to the production of a shorter soluble N-terminus (APPsβ) and the amyloidogenic C-terminal fragment of 99 residues (C99). C99, which contains the full amyloidogenic sequence of aminoacids, is then cleaved by γ-secretase, producing C-terminal 50 residues of APP, known as APP intracellular domain (AICD), and two forms of Aβ, Aβ40 and Aβ42 (Carlson, 2003, Evin et al., 2003, LaFerla and Oddo, 2005, Haass and Selkoe, 2007, Bateman et al., 2012) (Figure 1). While it is known that the major β-secretase responsible for APP processing is an integral membrane aspartyl protease called β-site APP-cleaving enzyme 1

(BACE1), γ-secreatase activity is more intricate. γ-secretase activity resides in a high molecular intramembraneous protease complex, consisting of four components: presenilin (PS), niscastrin, anterior pharynx defective 1(APH -1) and presenilin enhancer 1 (PEN-1). However it is known that PS constitutes the main active site (Vassar et al., 1999, Evin et al., 2003, Gandy, 2005, Blennow et al., 2006).

Although Aβ40 and Aβ42 are the main components of the senile plaques, the presence or progression of the plaques do not correlate with severity of the disease (Terry et al., 1991, Dickson et al., 1995, Shankar and Walsh, 2009). Moreover, it is now considered that the senile plaques are an end stage event of amyloid deposition, representing an inactive reservoir of species that are in equilibrium with the smaller assemblies of Aβ (Hardy and Selkoe, 2002). These smaller and soluble forms of Aβ are called oligomers and are believed to be the most toxic form of the peptide (Harper et al., 1997, Lambert et al., 1998, Walsh et al., 1999, Bitan et al., 2003, Hoshi et al., 2003). The monomeric form of Aβ (molecular weight ~ 4 kDa) that is produced from the proteolytic cleavage of APP, can undergo a conformational change to high β-sheet content, which is more prone to aggregate into soluble, short forms called oligomers and into larger insoluble fibrils, consequently developing into plaques (Blennow et al., 2006). Oligomers, such as dimers and trimers, can be more synaptotoxic compared to insoluble Aβ plaques since they can exert both intracellular and extracellular toxicity (Walsh and Selkoe, 2004, Haass and Selkoe, 2007, Benilova et al., 2012). Interestingly, experimental studies have demonstrated that Aβ oligomers show synaptotoxicity in hippocampal slice cultures and primary neuronal cultures, and *in vivo* in rodents they affect dendritic spine morphology and alter hippocampal long-term potentiation (LTP) and long-term depression (LTD) (Lambert et al., 1998, Walsh et al., 2002, Walsh and Selkoe, 2004, Cleary et al., 2005, Li et al., 2009, Wu et al., 2010, Jo et al., 2011). Moreover, these subtle synaptic alterations support that the toxic oligomeric form of Aβ and its intermediates are toxic as soon as they are produced, and are thought to play an important role in the early stages of the disease (Benilova et al., 2012).

The detrimental effects of oligomeric Aβ includes the deregulation of calcium (Ca^{2+}) homeostasis, oxidative stress and activation of an inflammatory response (Small et al., 2009, Demuro et al., 2010, Stutzmann and Mattson, 2011, Berridge, 2014, Lazzari et al., 2015). These events are the result of

the association of Aβ oligomers with the cellular membrane. By forming membrane pores (Jang et al., 2008, Jang et al., 2010a, Jang et al., 2010b, Sepulveda et al., 2010), disrupting membrane receptors function by intimate binding (Wang et al., 2004) or by interacting directly with the membrane (Verdier Y and B., 2004), Aβ oligomers can lead to an increase of Ca^{2+} levels within the cell that results in an accumulation of reactive species of oxygen (ROS) (Mutisya et al., 1994, De Felice et al., 2007, Huang et al., 2011) and activates the inflammatory response.

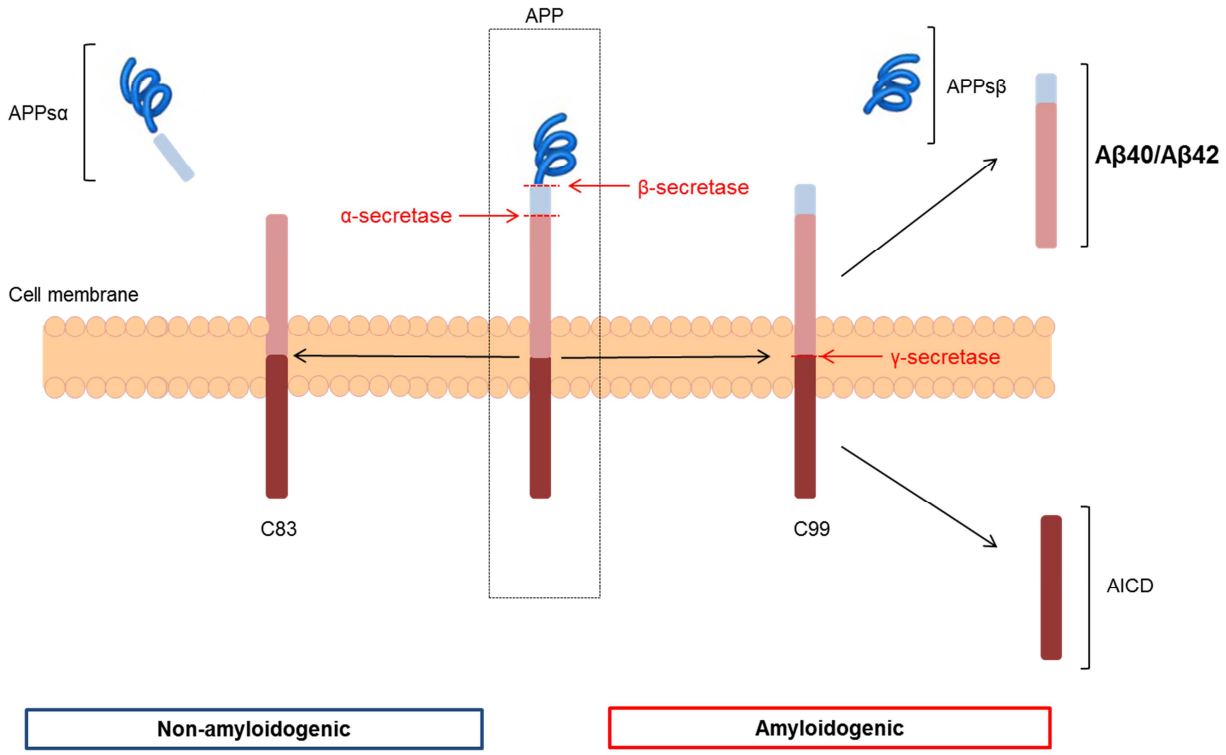

Figure 1. The proteolytic processing of amyloid precursor protein (APP). APP, a transmembrane protein, when cleaved by β- and γ-secretase leads to the production of Aβ peptides, in a process known as the amyloidogenic pathway. The two major forms of Aβ are Aβ40 and Aβ42, both of which are approximately 4 kDa. In the non-amyloidogenic pathway, APP is cleaved by α-secretase within the Aβ domain, thus preventing Aβ formation and producing the extracellular soluble APP-α peptides (APPsα).

1.2.1.2 Amyloid cascade hypothesis

The central hypothesis for the cause of AD was first described in 1984 by Glenner and Wong, when they identified that the basis of AD pathology was an imbalance between the production and clearance of Aβ (Glenner and Wong, 1984), which later led to the development of the amyloid cascade hypothesis. In normal conditions Aβ can be produced, degraded and cleared out of the brain. Aβ is normally degraded by the peptidases insulin-degrading enzyme (IDE) and neprilysin (NEP), and by endothelin-converting enzyme (Carson and Turner, 2002, Tezapsidis et al., 2009). Aβ can be cleared from the brain via receptor-mediated transcytosis in the endothelial cells of the BBB, through the low-density lipoprotein receptor-related protein (LRP), or by endocytosis involving the apolipoprotein E (ApoE) (Mackic et al., 1998, Donahue et al., 2006, Bell and Zlokovic, 2009, Tezapsidis et al., 2009).

Support for the amyloid cascade theory was achieved with the finding that AD could be inherited in a autosomal dominant manner (Goate et al., 1991). Indeed, mutations present in people with FAD are in genes that not only involve APP (Goate et al., 1991, Hardy and Higgins, 1992, Mullan et al., 1992), but also the presenilin enzymes (PS1 and PS2) (Sherrington et al., 1995, Scheuner et al., 1996), which undoubtedly affect Aβ production, in particular Aβ42 (Selkoe, 1991, Hardy and Higgins, 1992, Hardy and Selkoe, 2002, Blennow et al., 2006). Moreover, the observation that people with Down's syndrome have an extra APP gene, and that these individuals develop Aβ plaques early in life (Olson and Shaw, 1969), together with the fact that in families with FAD there is a duplication of the APP locus (Rovelet-Lecrux et al., 2006), led to the knowledge of a genetic framework for the amyloid hypothesis (Wisniewski et al., 1991, Selkoe, 2001). According to the amyloid cascade hypothesis, the mutations that occur in the APP gene or in the gene involving APP processing, are the main factors that will cause the imbalance between Aβ production and clearance. The net result is an increase in Aβ42 deposition that initiates cell death and leads to dementia (Hardy and Higgins, 1992, Hardy and Selkoe, 2002, Hardy, 2009, Karran et al., 2011). Indeed, cloning of PS1 and PS2 (Levy-Lahad et al., 1995, Sherrington et al., 1995) in cells and in transgenic mice lead to an increase in Aβ42 levels (Borchelt et al., 1996, Duff et al., 1996, Scheuner

et al., 1996), further supporting amyloid cascade hypothesis. However, the lack of correlation between dementia and plaque formation, which has thought to be the main reason for the cognitive decline, was brought an objection to the hypothesis. The number of amyloid deposits in the brain of an AD patient does not correlate well with the degree of cognitive impairment (Hardy and Selkoe, 2002). In fact, some individuals without symptoms of dementia may have cortical Aβ deposits. Moreover, it is now known that the soluble Aβ species appears to better correlated with cognitive decline (Näslund et al., 2000, Hardy and Selkoe, 2002). Such evidence led to the amyloid cascade hypothesis to be revisited and Aβ oligomers are now thought to be an important player in the pathology (Figure 2).

The amyloid cascade hypothesis now suggests that synaptotoxicity and neurotoxicity might be mediated by the oligomeric form of the peptide rather than insoluble plaques. Aβ oligomers will cause microglia and astrocytes activation and direct injury of synapses and neurones, which consequently alters the neuronal ionic homeostasis and causes oxidative stress, resulting in dysfunctional kinase activity. This dysfunctional enzyme activity will cause tau to be hyperphosphorylated, forming NFTs, and ultimately causing dementia (Hardy and Selkoe, 2002, Blennow et al., 2006, Karran et al., 2011) (Figure 2).

Another inclusion in the amyloid cascade hypothesis over the years was the discovery of ApoE gene (APOE) as major genetic risk factor for SAD (Corder et al., 1993, Strittmatter et al., 1993). The possession of an APOE allele 4 (APOE4) correlates with increased Aβ deposition and AD risk (Holtzman et al., 2000). This effect is gene-dose dependent: one allele 4 increases the risk of AD by 2-3 fold, whilst 2 copies of allele 4 increases the risk by 12 fold (Kim et al., 2009). APOE also encodes ApoE protein that is involved in the clearance of Aβ. It has been shown that the transport of Aβ across BBB through ApoE also can be affected by allele4, with APOE4 leading to the slowest transport, decreasing Aβ clearance out of the brain and initiating the same cascade of events that lead to dementia (Figure 2). However, the real mechanism for APOE4 and Aβ transport across the BBB remains unclear.

The amyloid cascade hypothesis includes several molecular process of the appearance of the pathology. It also includes the genetic background, as the autosomal dominant form for FAD, and

the genetic risk associated with APOE4. However it lacks of evidence in what concerns to the interaction of Aβ with tau (Karran et al., 2011). Moreover, the hypothesis is totally centred on the neurone as the main reason and origin of the pathology, whilst omitting interactions between the neurones and astrocytes (Demetrius et al., 2015) and other organelles important to the normal neuronal activity, such as mitochondria.

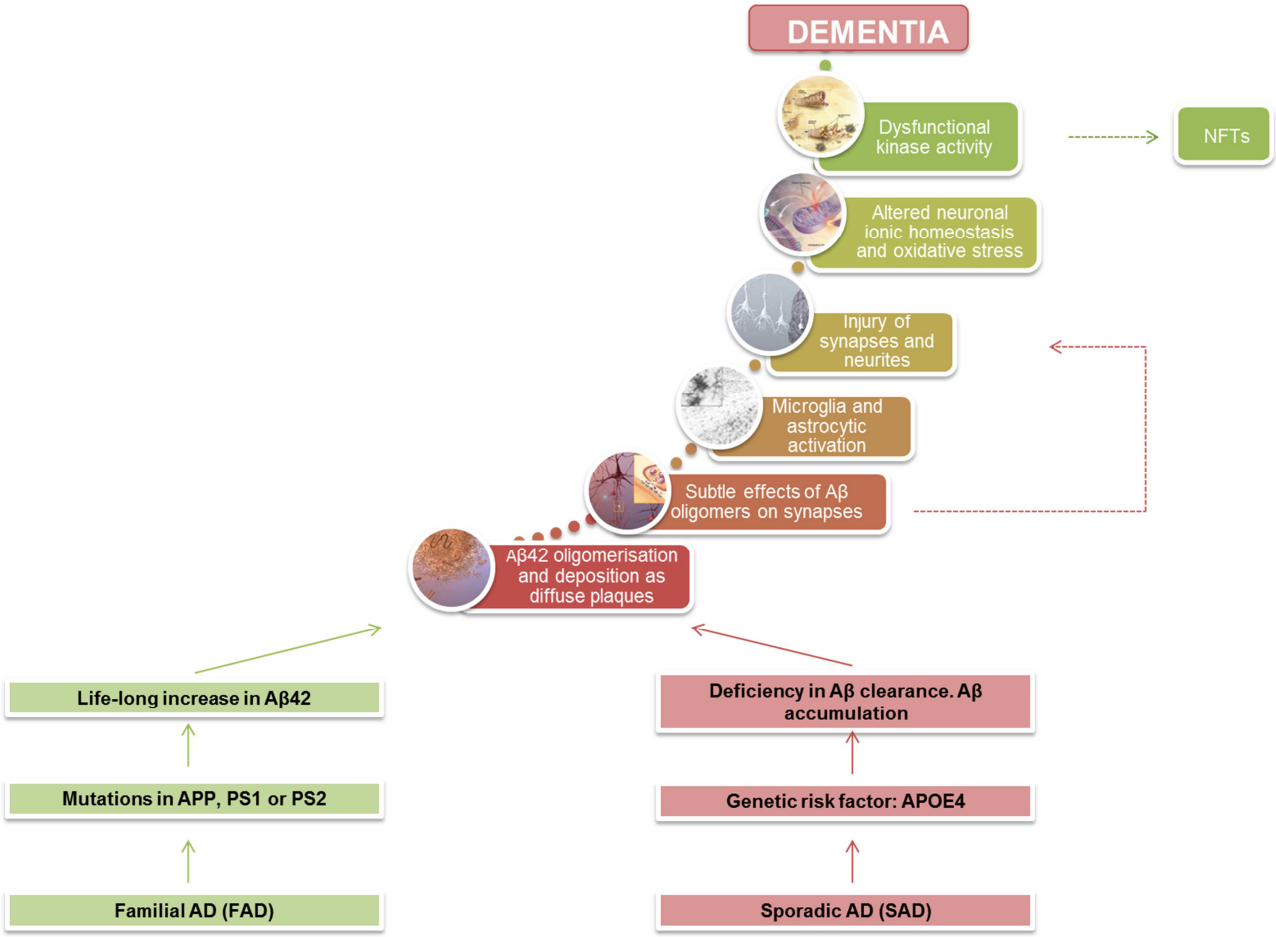

Figure 2. Amyloid cascade hypothesis. The amyloid cascade hypothesis is considered as a gene-centric model for both familial and sporadic forms of AD. It postulates that the imbalance between Aβ production and clearance is due to genetic mutations (APP gene or in genes involved in APP processing, associated with the familiar form of the disease (Demetrius et al., 2015)) or genetic factors (e.g. APOE4 as seen in SAD). In both forms of AD, an accumulation of Aβ42 occurs, due to an increased production in the familial form or a decrease in clearance in the sporadic form. Aβ oligomers can directly inhibit hippocampal LTP and LTD and therefore impair synaptic function. The formation of plaques also leads to the activation of the innate immune system and initiation of an inflammatory response characterised by activation of microglia and astrocytes, which causes progressive synaptic and neuritic injury. Aβ oligomers can also directly cause injury in synapses and neurites. Such alterations lead to changes in the neuronal ionic homeostasis, in particular Ca^{2+}, and oxidative balance, which affects kinase activity, leading to the formation of NFTs and ultimately dementia. AD – Alzheimer's disease, Aβ – amyloid β protein, APP – amyloid precursor protein, SAD – sporadic form of AD, APOE4 – ApoE gene allele 4, LTP – long-term potentiation, LTD – long-term depression, Ca^{2+} - calcium, NFTs – neurofibrillary tangles.

1.2.1.3 Mitochondrial cascade hypothesis

As mentioned above, the amyloid cascade hypothesis is based on genetics and explains the molecular pathways for the FAD, however it does not specify what triggers the common late-onset form of the disease, the SAD. The mitochondrial cascade hypothesis was first proposed in 2004 by Swerdlow and Khan in order to explain the molecular basis of SAD (Swerdlow and Khan, 2004). This theory was built on the idea that mitochondrial DNA (mtDNA) inheritance would have an impact on AD development, through the effects of age and time on mitochondrial function (Parker et al., 1989, Swerdlow et al., 2014), suggesting that mitochondrial dysfunction is the primary event that leads to Aβ deposition and synaptic degeneration (Moreira et al., 2010) (Figure 3).

AD patients have increased levels of sporadic mutations in mtDNA (Corral-Debrinski et al., 1994, Hirai et al., 2001) and there is around a 3-fold increase in oxidative damage in mtDNA of AD patients which might cause the mutations (Wang et al., 2014). mtDNA is highly vulnerable to oxidative damage since it does not have protective proteins, such as histone, and its repair system is close to the site of ROS generation within the mitochondria (Wang et al., 2014), therefore is clear that mtDNA mutations can increase oxidative stress and ROS production. In fact, many of these mutations occur in sites of mtDNA transcription and replication which results in reduced transcription levels of mitochondria proteins and ultimately affects mitochondria function (Wallace et al., 1997, Coskun et al., 2004).

Age-associated changes in mitochondria are known to affect the mitochondrial electron transport chain (ETC), the main responsible for the cell redox state (Chance et al., 1979, Shigenaga et al., 1994, Swerdlow and Khan, 2004), leading to ETC decline and increased oxidative stress (Miquel et al., 1980, Trounce et al., 1989, Cooper et al., 1992, Ojaimi et al., 1999, Sastre et al., 2000, Lenaz et al., 2002, Barja, 2014). Moreover, increase oxidative stress has been implicated in the aging process (Wang et al., 2014) which can also affect mtDNA, and markers of oxidative stress have been also found in AD brains (Smith et al., 1995, Markesbery, 1999, Reddy, 2006, Chaturvedi and Beal, 2008, Reddy and Beal, 2008).

Mitochondria are energy-generating organelles that play an important role at chemical synapses, due to their high energy demand (Cheng et al., 2010). Mitochondria are responsible for the generation of adenosine trisphosphate (ATP), Ca^{2+} regulation, modulation of caspase activity and stimulation of oxidative phosphorylation (Calkins et al., 2012). Mitochondrial function and integrity are crucial for synaptic function, since all the ATP consumed by neurones is entirely used for cellular and synaptic function (Calkins et al., 2012). Moreover, it is known that changes in synaptic function and in mitochondrial integrity occur early in AD pathology, even before Aβ accumulation, and are associated with cognitive decline (Du et al., 2010, Reddy et al., 2010).

Increased in ROS through aging, environment factors and mtDNA mutations will cause impairment in mitochondria function. Damaged and dysfunctional mitochondria will then be transported to synaptic terminals where they will produce low ATP levels and that will ultimately lead to synaptic damage (Reddy and Beal, 2008), which can in turn produce more oxidative stress. This interaction between mitochondria dysfunction and oxidative stress forms a vicious cycle that amplifies the deficits. In the first instance, neurons will try to compensate by pushing their bioenergetics infrastructures, such as increases in oxidative glucose metabolism; overexpression of cytochrome oxidase and try to control mitochondrial dynamics, which is known to contribute to an increase in Aβ production and accumulation (Manczak et al., 2004, Youle and van der Bliek, 2012, Demetrius and Driver, 2013, Swerdlow et al., 2014). As mitochondrial decline continues, neurones will no longer be able to compensate and will eventually degenerate (Swerdlow and Khan, 2004, 2009, Swerdlow et al., 2014).

Studies have demonstrated that Aβ, in particular monomers and oligomers, can associate with mitochondrial membranes (Caspersen et al., 2005, Crouch et al., 2005, Devi et al., 2006, Hansson Petersen et al., 2008, Yao et al., 2009, Manczak and Reddy, 2012). This association can interfere with mitochondrial proteins involved in mitochondrial dynamics (fusion and fission) and transport (Lustbader et al., 2004, Du et al., 2008, Wang et al., 2008, Wang et al., 2009, Manczak et al., 2010, Calkins and Reddy, 2011, Manczak et al., 2011, Manczak and Reddy, 2012). Aβ is also known to disrupt the ETC, which increases the production of ROS and inhibits the generation of cellular ATP, leading to oxidative stress and mitochondrial dysfunction (Reddy and Beal, 2008, Reddy, 2009).

APP is also found in both mitochondrial inner membrane and in mitochondrial matrix (Swerdlow et al., 2010), which suggest that mitochondria can also produce Aβ. Moreover, it has been shown that mitochondrial dysfunction pushes APP processing towards Aβ production (Gabuzda et al., 1994, Gasparini et al., 1997, Webster et al., 1998), which supports the mitochondrial cascade hypothesis as mitochondria a primary event towards Aβ production and degeneration.

Therefore, in the amyloid cascade hypothesis the production of Aβ is the primary event in the autosomal dominant form of the disease (FAD), leading to secondary mitochondrial dysfunction. In the mitochondrial cascade hypothesis, mitochondrial dysfunction is thought to be the key reason for pathological hallmarks and Aβ formation in the development of SAD (Swerdlow and Khan, 2004, 2009). Moreover, mitochondrial dysfunction has also been shown to produce other AD-associated characteristics aside oxidative stress, such as tau phosphorylation and inflammation (Blass et al., 1990, Ichimura et al., 2003, Szabados et al., 2004) (Figure 3).

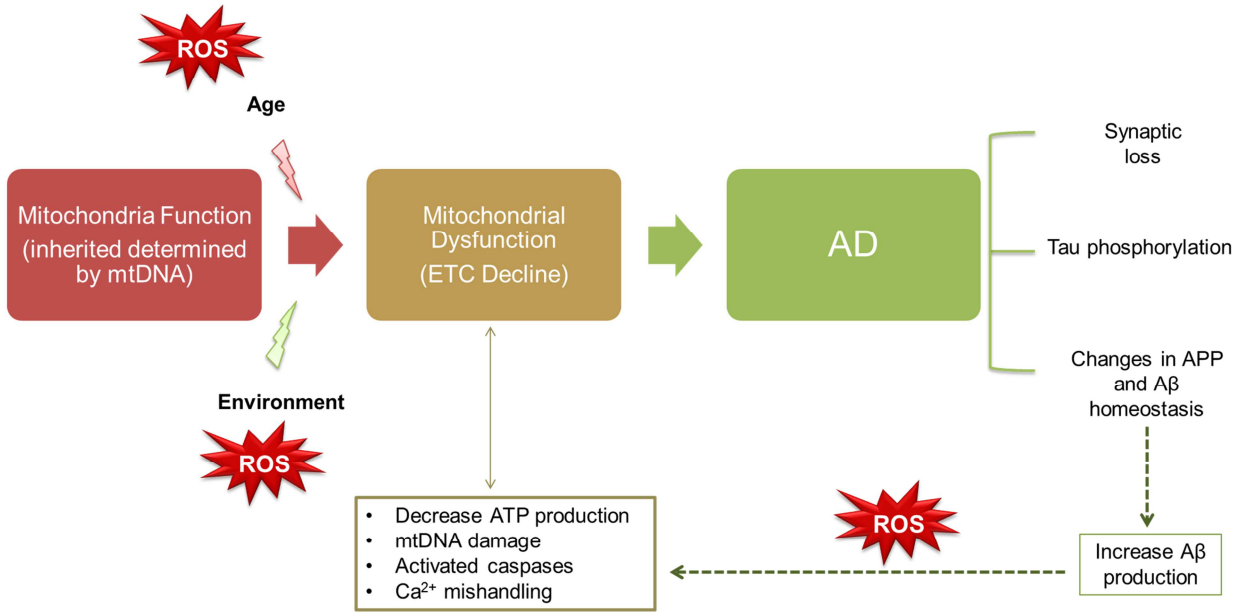

Figure 3. Mitochondrial cascade hypothesis. The mitochondrial cascade hypothesis states that in sporadic, late-onset AD, mitochondrial function will be the primary event that will lead to Aβ production and accumulation. According with the hypothesis, each individual has an inherited determined mitochondrial capacity/function, defined by its mtDNA that can be affected by the aging process and environment (such as reactive species of oxygen, ROS). Both age and environment lead to alterations in electron transport chain (ETC) causing its dysfunction. An impairment of the ETC will affect mitochondria function and result in decreased ATP production, mtDNA damage, activation of caspases and deregulation of Ca^{2+} homeostasis. Initially, neurones will try to compensate this decline in mitochondrial function by increasing oxidative glucose metabolism, overexpressing cytochrome oxidase and by increasing or decreasing mitochondrial dynamics and transport. During this period, Aβ production increases and accumulates. In turn, the Aβ that starts to accumulate will generate even more ROS and will interact with mitochondria, causing even more mitochondrial dysfunction, creating a vicious cycle. However, as mitochondrial decline progresses neurones are no longer able to cope or compensate and synaptic degeneration starts.

1.2.2 Non-genetic risk factors for Alzheimer's disease

The familial form of AD (FAD) is known to be caused by mutations in single genes. Although it is known that some genes such a APOE4 can lead to an increased risk of late-onset/SAD, other risk factors such as environmental are thought to play a key role in the development of this form of AD (Qiu et al., 2009, Morris et al., 2014). Below are some of the known risk factors for developing AD.

1.2.2.1 Age and gender

Age is one of the greatest risk for AD, although AD is not a normal part of aging process (Alzheimer's Association Report, 2014). The majority of cases are diagnosed in people aged above 65 years and its prevalence almost doubles every 5 years after this age (Qiu et al., 2009, Reitz and Mayeux, 2014). However, another important factor for the development of AD is sex. Epidemiological studies report that more women than men developing AD (Ott et al., 1998, Sinforiani et al., 2010, Viña and Lloret, 2010) and gender differences in mortality are present as women live longer than men after diagnosis of AD (Sinforiani et al., 2010, Wattmo et al., 2011, Stevens and Brown, 2015).

1.2.2.2 Blood pressure

High blood pressure during midlife has been associated with increased risk for developing AD later in life (Swan et al., 1998, Launer et al., 2000, Kivipelto et al., 2001, Whitmer et al., 2005b, Reitz and Mayeux, 2014). It is believed that hypertension causes cognitive impairment due to its effect on cerebrovascular integrity and changes in the BBB (Reitz and Mayeux, 2014), leading to protein extravasation into the brain tissue (Kalaria, 2010), which causes cell damage, reduction in neuronal and synaptic function and increases in Aβ accumulation, since Aβ transporters responsible for Aβ clearance are present at the BBB (Deane et al., 2004). Anti-hypertensive drugs have demonstrated to be protective against AD and dementia (Qiu et al., 2005, Khachaturian et al., 2006, Qiu et al., 2009), which supports the role of blood pressure as a risk factor for the disease. However, as age progresses the effect of high blood pressure as a risk factor for AD decreases, and often AD

patients have low blood pressure and high blood pressure is seen as a protective factor later in life (Reitz and Mayeux, 2014). This reduction of blood pressure in AD patients can be caused by vessel stiffening, weight loss and changes in blood flow, that normally occur after the onset of AD (Reitz and Mayeux, 2014).

1.2.2.3 Diabetes

Several epidemiological studies have demonstrated that diabetes and insulin resistance are risk factors for developing AD (Leibson et al., 1997, Ott et al., 1999, Luchsinger et al., 2001, Peila et al., 2002, Arvanitakis et al., 2004, Janson et al., 2004, Akter et al., 2011, Hölscher, 2011, Talbot et al., 2012, Umegaki, 2012, Ninomiya, 2014), in particular type 2 diabetes (T2D) has been shown to double the risk for AD (Leibson et al., 1997, Luchsinger et al., 2001, Peila et al., 2002).

T2D is characterised by increased levels of blood glucose. This increase in blood glucose is due to three main events: increased hepatic glucose production; impaired insulin production by the pancreatic β-cells; and insulin resistance (Akter et al., 2011, Butterfield et al., 2014). Insulin resistance occurs when there is a reduced responsiveness of the cell or organism to insulin (Shanik et al., 2008). In T2D there is an overproduction of insulin, leading to hyperinsulinemia (Festa et al., 2006), that results in this resistant state, since insulin receptors become less sensitive to insulin.

Insulin can cross the BBB and play an important role in the brain. Moreover, it is known that chronic exposure to high levels of insulin, due to peripheral hyperinsulinemia, has a negative effect in BBB function and insulin receptors activity, leading to a decrease of insulin transport into the brain (Strachan et al., 1997). Chronic peripheral hyperinsulinemia is associated with a specific pattern where insulin levels in the brain are initially high but then with the persisted exposure to insulin the insulin transport to the brain across the BBB gets down-regulated, leading to decreased brain insulin levels and impaired insulin signalling (Banks et al., 1997, Cholerton et al., 2013). Interestingly, it has been shown that long-term neuronal exposure to high levels of insulin can lead to neuronal degeneration and have an impact on cognitive function (Son et al., 2011, Blázquez et al., 2014a, Dineley et al., 2014, Ma et al., 2015). Insulin receptors are thought to be involved in the

regulation of synapses including dendritic sprouting, and insulin itself is known to facilitate attention and memory in humans (Holscher, 2014). In addition, brain insulin resistance has been demonstrated in particular in the hippocampus, prior to cognitive decline in AD patients (Talbot et al., 2012). This supports that memory impairment associated with AD and T2D could be due to impairments in insulin signalling.

Insulin also influences Aβ metabolism and is known to increase Aβ deposition and tau phosphorylation (Park, 2001, Reitz and Mayeux, 2014). In the initial disease stage of T2D, hyperinsulinemia induces Aβ accumulation through competition with Aβ for IDE (Gasparini et al., 1997, Farris et al., 2003). In normal conditions Aβ is mainly degraded by IDE, which is also responsible for insulin degradation in both neurones and microglia (Sims-Robinson et al., 2010). However, the affinity for insulin is higher than for Aβ, which means that in T2D the increase levels of insulin within the brain will result in less Aβ clearance through IDE and increased Aβ accumulation (Farris et al., 2003, Ma et al., 2015). This supports that T2D could lead to AD and explains why T2D patients have memory impairments (Stewart and Liolitsa, 1999, Gold et al., 2007).

Interestingly, in AD brains patients the levels of IDE as well its activity are reduced (Cholerton et al., 2013) and hyperinsulinemia is seen (Frölich et al., 1998). Studies have demonstrated that AD patients present low levels of insulin in the brain and cerebrospinal fluid (CSF) but high levels in the plasma, as seen in diabetic patients, which is related to impaired insulin transduction (Craft et al., 1998, Frolich et al., 1999, Ghasemi et al., 2013). The high levels of plasma insulin are known to be a consequence of insulin resistance, whilst decrease CSF insulin levels may be due to decreased insulin clearance (which in AD will be decrease due to high levels of Aβ competing with IDE) and/or decreased insulin uptake at the BBB (Craft et al., 1998, Frolich et al., 1999, Ghasemi et al., 2013). Moreover, insulin receptors function and expression deteriorate with the progression of the disease in AD patients (Rivera et al., 2005) and in post-mortem brain deficits in insulin signalling are present (Steen et al., 2005, Talbot et al., 2012). Since impaired insulin sensitivity has been linked to cognitive deficits (Benedict et al., 2012) this suggest that insulin-related signalling may play an important role in learning and memory and impairment (De Felice and Ferreira, 2014). Moreover, it shows that persistently high levels of insulin may conversely negatively influence memory through

desensitization of insulin signalling in the brain. Indeed, insulin receptors are present in the hippocampus and entorhinal cortex, brain regions known to be affected in AD and are responsible for learning and memory (Reitz and Mayeux, 2014). Studies have shown that once in the brain insulin can modulate synaptic function (Wan et al., 1997, Gasparini et al., 2001, Skeberdis et al., 2001, van der Heide et al., 2006, Jin et al., 2011) and control synaptic density in vivo (Chiu et al., 2008). In rodents studies have demonstrated that insulin contributes to long-term memory consolidation and to improve spatial learning and memory (Zhao et al., 2004, Haj-ali et al., 2009, McNay et al., 2013, Vandal et al., 2014) In addition, it has been shown that insulin can have beneficial actions on memory through intranasal administration in early AD patients, by restoring insulin levels and improving cognitive performance (Craft et al., 2012, Freiherr et al., 2013).

1.2.2.4 Obesity

Obesity, in particular midlife obesity (between 40 and 60 years old), has been associated with increased risk for AD in several epidemiological studies (Gustafson et al., 2003, Kivipelto et al., 2005, Rosengren et al., 2005, Whitmer et al., 2005a, Whitmer et al., 2008, Fitzpatrick et al., 2009, Hassing et al., 2009, Profenno et al., 2010, Anstey et al., 2011, Xu et al., 2011, Gustafson, 2012, Anstey et al., 2014, Tolppanen et al., 2014). While being overweight increases the risk of developing AD by 35%, being obese (defined as body mass index (BMI) > 30 kg/m^2) increases the risk by 75% (Whitmer et al., 2005a).

Higher BMI is also associated with structural brain changes (Gustafson et al., 2004, Gazdzinski et al., 2008), such as loss of neurones and myelin in brain regions, such as hippocampus, that are affected in AD (Raji et al., 2010, Mueller et al., 2012). In addition, obesity has also been associated with several other processes that are also observed in aging and AD, such as increased oxidative stress, inflammation and insulin resistance (Dandona et al., 2005, Lumeng et al., 2007, Strissel et al., 2007, Bhat, 2010, Nguyen et al., 2014).

1.2.3 Experimental animal models of Alzheimer's disease

Over the years the need to understand the mechanism of AD has led to the development of genetic mouse models. Although most cases of AD are sporadic, the knowledge of specific genetic mutations in the familial form gave enough background for the design of transgenic mice (Tg). Over 130 mutations have been identified in FAD, with over 20 of these in APP (Gotz and Ittner, 2008), but only a few have been expressed in transgenic mice, such as V717I "London" mutation (Goate et al., 1991), V717F "Indiana" mutation (Murrell et al., 1991), K670D/M671L "Swedish or APPswe" (Mullan et al., 1992) and E693G "Artic" mutation (Nilsberth et al., 2001). However, since it is known that the presence of APOE4 allele is a genetic risk factor for AD, ApoE4 transgenic mice have also been developed and show memory impairments (Raber et al., 1998, Hartman et al., 2001).

The most widely used transgenic mouse models of AD express human APP or PS mutations, which result in age-dependent Aβ accumulation into extracellular plaques (LaFerla and Green, 2012) and memory deficits (Hall and Roberson, 2012). However, these mice do not develop NFTs (LaFerla and Green, 2012). Although double transgenic mice, expressing APP and PS mutations, such as APP/PS1 (Holcomb et al., 1998) and APP/PS2 (Richards et al., 2003) have an accelerated AD-phenotype and memory deficits, they still do not present with tau pathology. Other transgenic mice have been created with tau mutations that are known to cause frontotemporal dementia (FTD), but although these mice develop NFTs, tau mutations are not known to be associated with AD (Lewis et al., 2001, Hall and Roberson, 2012). However, in order to recapitulate the two pathological features of AD, the triple transgenic mouse model of AD (3xTgAD), which has mutations in APP, PS1 and tau, was created.

1.2.3.1 3xTgAD mouse model

Single and double transgenic mice have failed to fully recapitulate the entire neuropathology of AD (Wong et al., 2002), showing that expressing just one hallmark of AD (Aβ or tau) is not enough to trigger the development of other signature lesion (tau or Aβ) (Oddo et al., 2003b). Developed by La Ferla and colleagues, the 3xTgAD model harbouring three mutant genes: PS1M146V, APPSwe,

and tauP301L, was the first to develop both plaque and tangle pathology in the specific brain regions associated with AD pathology in humans (Oddo et al., 2003a, Oddo et al., 2003b). In this mouse model, extracellular Aβ deposits precede tau pathology, consistent with the amyloid cascade hypothesis, and exhibit deficits in synaptic plasticity, including LTP, that occur before plaque deposition and NFTs formation (Oddo et al., 2003a, Oddo et al., 2003b). The role of the key three mutations are important for the development of the neuropathology, however it is also known that PS1M146V mutation can influence multiple signalling pathways and have a more global effect on neuronal function and plasticity and cause synaptic loss (Thinakaran et al., 2004) whilst tauP301L mutation is known to result in reduced activity and progressive motor impairment in mice (Terwel et al., 2005), which can both affect the behaviour outcome.

Intraneuronal Aβ is first detected at 3-4 months of age in 3xTgAD mice in the neocortex, followed by the CA1 and CA3 of the hippocampus at 6 months (Oddo et al., 2003a). However, other studies have demonstrated the presence of intraneuronal Aβ as early as 2 (Mastrangelo and Bowers, 2008) or 4 months in the hippocampus (Billings et al., 2005). Extracellular Aβ plaques only start to appear at 6 months in layers 4 and 5 of the neocortex (Oddo et al., 2003a) and by 12 months in the hippocampus and other cortical regions (Giménez-Llort et al., 2007). NFTs are not detected until 12 months of age in the CA1 region of the hippocampus, which then progressively affect neurones in the cerebral cortex in older mice (Oddo et al., 2003a).

Previous data from our lab have shown that there are intraneuronal Aβ-positive cells in cortex, hippocampus and amygdala from 4 months of age in 3xTgAD mice (Knight et al., 2013), and that Aβ plaques were not visible until approximately 12-months-old in the hippocampus, starting at the dorsal subiculum, (Knight et al., 2012, Knight et al., 2014), whilst hyperphosphorylated tau positive cells were not detected until 7 months (Knight et al., 2014).

Inflammation is also a key event in AD pathology, and the 3xTgAD mice also show activation of astrocytes and microglia (Janelsins et al., 2005, Chen et al., 2011, Kamphuis et al., 2012, Knight et al., 2014). In our lab we have demonstrated that in 3xTgAD mice microglia activation occurs from 15 months of age in the hippocampus, in particular in the dorsal subiculum, in areas associated to Aβ plaques (Knight et al., 2014).

3xTgAD also present with cognitive impairments, the main behavioural symptom of AD. In AD patients, tests of working memory can predict the progression and severity of the disease (Stevens and Brown, 2015). In animal models, behavioural tests are also used to assess memory impairment. Such behavioural analysis has demonstrated that 3xTgAD mice have reduced exploratory behaviour and movement, normally assessed in open-field tests, as well learning and memory deficits at 6 months of age, and that these behavioural changes precede Aβ plaque formation (Billings et al., 2005, Giménez-Llort et al., 2007, Sterniczuk et al., 2010). Higher levels of anxiety during open-field, elevated plus maze, passive avoidance and horizontal ladder tests have also been observed in 3xTgAD mice (Sterniczuk et al., 2010).

Some studies assessing spatial reference memory, using the Morris Water Maze (MWM), have found no deficits at 2 months of age (Billings et al., 2005, Billings et al., 2007, Clinton et al., 2007, Giménez-Llort et al., 2007, McKee et al., 2008) however working memory is impaired at this age in 3xTgAD mice (Stevens and Brown, 2015). Studies have also demonstrated that 3xTgAD mice have a specific decline in episodic-like memory, an hippocampal-dependent memory, from 3 months old, that becomes a deficit by 6 months of age (Davis et al., 2013a, Davis et al., 2013b). In our previous studies we have demonstrated that 3xTgAD exhibit hippocampal-dependent deficits by 7-8 months of age, assessed by the Y-maze spontaneous alternation and novel object recognition (NOR) tests (Knight et al., 2014), reduced general exploratory behaviour in open-field by 3-4 months (Knight, 2010) and deficits in smell recognition and MWM tests as early as 3 months of age (Knight et al., 2014). These evidences show that there is no consistency to when exactly the memory deficits appear in 3xTgAD mice, suggesting that the results depended on the cohort and behaviour test used and it should be taken into account in experimental studies.

1.3. Obesity

1.3.1 Obesity and the adipose tissue

Obesity is a condition defined by an increase in fat content and occurs when the energy intake is greater than energy expenditure (Gesta et al., 2007). According to the World Health Organization (WHO) in 2014 more than 1.9 billion adults were overweight and over 600 million obese. This increase in obesity, which doubled between 1980 and 2014, is due to an increase consumption of diets high in fat, such as western diets (WHO, 2014). Obesity is associated with increased risk for other disorders, such as T2D, hypertension, stroke and AD (Haslam and James, 2005, Beydoun et al., 2008, Anstey et al., 2014).

Fat storage occurs in a mesodermal tissue, called adipose tissue, and its distribution defines the metabolic risk associated with obesity (Gesta et al., 2007). Intra-abdominal/visceral accumulation of adipose tissue, defined as central or apple-shaped obesity, is associated with increased risk for metabolic diseases. On the other hand, peripheral or pear-shaped obesity, characterised by fat accumulation in thighs and hips, is associated with no risk or little for metabolic disorders (Kissebah and Krakower, 1994, Gesta et al., 2007). Therefore, the main type of obesity that is associated with other comorbidities and diseases is central obesity.

Adipose tissue is divided in two types: white adipose tissue (WAT) and brown adipose tissue (BAT). In humans, WAT is distributed throughout the body with main depots in the intra-abdominal area, around the omentum, intestines and perirenal areas, as well in subcutaneous depots in abdomen, thighs and buttocks (Gesta et al., 2007). WAT is also involved in metabolism control through energy homeostasis, adipocyte differentiation and insulin sensitivity (Balistreri et al., 2010). WAT is the primary site of triglyceride/energy storage representing the major source of free fatty acids (FFAs), whilst BAT is responsible for both basal and inducible energy expenditure in the form of thermogenesis (Gesta et al., 2007). Thermogenesis is an important process by which dissipation of energy produces heat, and occurs through the expression of the uncoupling protein-1 (UCP-1), a protein found in the inner mitochondrial membrane (Cannon and Nedergaard, 2004, Saely et al.,

2012). In humans it is now known that BAT is present in cervical, supraclavicular, axillary and paravertebral regions (Nedergaard et al., 2007), whilst in rodents it is mostly concentrated in the interscapular region (Nedergaard et al., 2007).

Adipose tissue is composed of adipocytes that differ in a morphological point of view from other types of cells due to the presence of large lipid droplets (Gesta et al., 2007). Although both WAT and BAT adipocytes arise from mesenchymal/mesodermal stem cells from preadipocytes, mature adipocytes from WAT and BAT have distinct markers. WAT is characterised by the presence of leptin whilst BAT has UCP-1 (Gesta et al., 2007). Morphologically WAT and BAT are also different. As the name suggest, WAT is distinguish by the presence of white adipocytes, whilst BAT has smaller number of fat cells rich in mitochondrial chromogens, which is responsible for the brown colour of the tissue (Redinger, 2009).

In obesity adipocyte necrosis is increased, both in humans and in mice (Gesta et al., 2007), whilst BAT mass and function decreases (Redinger, 2009). WAT is known to become inflamed in obese conditions, characterised by a cellular WAT composition remodelling: adipocytes increase in number (hyperplasia) and size (hypertrophy), there is infiltration of macrophages and fibrosis occurs (Jernås et al., 2006, Maury et al., 2007, Skurk et al., 2007, Bourlier et al., 2008, Henegar et al., 2008, Spalding et al., 2008, Balistreri et al., 2010). Moreover, during obesity WAT cells are exposed to increased stress due to increase metabolic overload, affecting mainly mitochondria (Houstis et al., 2006, Hotamisligil and Erbay, 2008). As a result, more ROS is produced by expanded WAT and, in addition to inflammation (Balistreri et al., 2010, Miller and Spencer, 2014, Nguyen et al., 2014, Spielman et al., 2014, Tucsek et al., 2014a), oxidative stress also occurs (Keaney et al., 2003, Furukawa et al., 2004, Houstis et al., 2006, Tucsek et al., 2014a).

1.3.2 Obesity and systemic inflammation

It is known that obesity can induce chronic low-grade systemic inflammation (Hotamisligil et al., 1995, Weisberg et al., 2003, Wellen and Hotamisligil, 2005, Gregor and Hotamisligil, 2011), which is believed to originate within WAT (Guri and Bassaganya-Riera, 2011). In fact, WAT is able to

produce pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α). Obese individuals have increased levels of circulating biomarkers of inflammation, such as TNF-α, interleukin-6 (IL-6) and the acute phase response protein C-reactive (CRP) (Qatanani and Lazar, 2007). This pro-inflammatory state is thought to be due to the dietary profile of obese individuals. It is known that the FFAs that high-fat diets contain, in particular saturated fatty acids (SFAs), can initiate this inflammatory response, since they can stimulate lipopolysaccharide (LPS) receptor and toll like receptor 4 (TLR4) (Shu et al., 2012, Miller and Spencer, 2014). Moreover, obesity is known to lead to macrophage accumulation in WAT, in particular visceral WAT (Balistreri et al., 2010), apoptosis of adipocytes and reduced WAT vascularity. These events contribute even more to further inflammation (Weisberg et al., 2003, Xu et al., 2003, Shu et al., 2012, Miller and Spencer, 2014), increasing the level of circulating pro-inflammatory cytokines and other inflammatory markers, defined as adipokines (Gesta et al., 2007, Balistreri et al., 2010). The rise of cytokines and adipokines will eventually affect the liver and muscle and contribute to insulin resistance (Xu et al., 2003, Shu et al., 2012). Interestingly, the inflammatory profile observed in obese individuals (increased TNF-α, IL-6 and CRP), are also seen in individuals who are insulin resistance, and can predict the development of T2D (Qatanani and Lazar, 2007). Several studies have demonstrated the link between the endocrine and inflammatory mechanisms of insulin resistance in obesity (Qatanani and Lazar, 2007). TNF-α is known to activate serine kinases, which directly or indirectly can phosphorylate insulin receptor substrate (IRS) 1 and 2, resulting in a decrease ability of insulin to stimulate phosphotidylionositol-3 kinase (PI3K)-dependent pathways that normally result in a glucose uptake and metabolism (Hirabara et al., 2012). In addition, in obese individuals who present high levels of circulating FFAs, FFAs are known to activate protein kinase C (PKC), which activates serine/threonine kinases that inhibit insulin signalling and TLR4 (Qatanani and Lazar, 2007). Therefore FFAs can also trigger insulin resistance.

Another important feature that is associated with obesity induced-inflammation is oxidative stress. Activated immune cells can generate large amounts of ROS, and in turn pro-inflammatory cytokines can promote ROS production (Miller and Spencer, 2014). Moreover, the chronic high

levels of FFAs and lipids that characterise obese individuals, are also associated with deleterious pathways such as generation of ROS and mitochondrial dysfunction (Qatanani and Lazar, 2007).

1.3.3 Central inflammation: obesity in the brain

In addition of peripheral inflammation, obesity has been associated with central inflammation and has a detrimental effect in the brain, in particular within the hypothalamus. Hypothalamic inflammation has been shown in several studies where high-fat diets increase the levels of pro-inflammatory cytokines and activate the pro-inflammatory transcription factor nuclear factor κB (NFκB) in this brain region (Souza et al., 2005). This state of inflammation leads to activation and proliferation of microglia (the brain macrophages) in the hypothalamus, leading to a further increase in cytokines within the brain, which has been demonstrated to disrupt leptin and insulin signalling, leading to brain insulin resistance (Qatanani and Lazar, 2007, Milanski et al., 2009, Posey et al., 2009, Thaler et al., 2012). This increase in pro-inflammatory cytokines in the hypothalamus in response to a high-fat diet leads to an increase in the suppressor of cytokine signalling 3 (SOCS3) proteins, which phosphorylate IRS and represses the leptin-receptor through the activation of the signal transducer and activator of transcription 3 (STAT3) pathway. SOCS proteins function as feedback inhibitors of the janus kinase (JAK) and signal transducer and activator of transcription (STAT) signalling pathway and they can terminate innate and adaptive immune responses and recent studies have demonstrated the negative regulatory function of SOCS3 in microglia and astrocytes (Qin et al., 2012). Moreover, SOCS3 pathway is involved in leptin (through phosphorylation of IRS) and insulin (repression of STAT3) signalling, which leads to reduced insulin sensitivity (Howard and Flier, 2006, Miller and Spencer, 2014), and loss of SOCS3 *in vivo* has shown to have profound effects on inflammation, fat-induced weight gain and insulin sensitivity (Carow and Rottenberg, 2014).

As previous described, insulin has an important role in synaptic plasticity and memory (Watson and Craft, 2004) and insulin receptors are highly expressed in brain regions involved in memory and learning, such as hippocampus (Zhao and Alkon, 2001, Naderali et al., 2009). Therefore, insulin

resistance induced by inflammation in obesity can affect cognitive function. Central inflammation has been observed within the hippocampus of mice fed with a high-fat diet, showing elevated TNF-α levels (Jeon et al., 2012) and microglia markers (Jeon et al., 2012, Knight et al., 2014). Moreover, central inflammation has been demonstrated to disrupt the internal hypothalamic circuit and potential hypothalamic outputs to other brain regions that are involved in cognitive function, such as hippocampus (Miller and Spencer, 2014). Indeed, is it known that the neuronal system that is involved in cognition and memory is affected in obesity (Stranahan and Mattson, 2012). Clinical studies have demonstrated that there is a positive association between inflammation and cognitive decline in obesity (Sweat et al., 2008, Sellbom and Gunstad, 2012, Wichmann et al., 2014).

1.3.4 Obesity and the brain: a bridge to Alzheimer's disease

Obesity appears to have a detrimental effect not only on metabolism but also in brain structure. Obesity has been associated with neuronal and/or myelin abnormalities, in grey and white matter (Gazdzinski et al., 2008, Naderali et al., 2009, Gazdzinski et al., 2010, Mueller et al., 2012), brain atrophy in several studies (Enzinger et al., 2005, Ward et al., 2005, Taki et al., 2008, Raji et al., 2010, Fotuhi et al., 2012, Brooks et al., 2013) and reduced focal grey matter volume (Pannacciulli et al., 2006). In particular, the hippocampus and frontal lobes appear to be highly sensitive to obesity-induced structural alterations, with studies demonstrating a positive correlation between BMI and grey matter volume in these regions (Pannacciulli et al., 2006, Taki et al., 2008, Raji et al., 2010). Hippocampal volume is associated with cognitive function (Stewart et al., 2005) and a reduction in volume can predict cognitive decline and dementia (Elias et al., 2000). Moreover, midlife obesity leads to hippocampal atrophy and executive function decline (Debette et al., 2011), which supports the fact that midlife obesity is associated with greater risk for developing dementia, in particular AD.

The relationship between midlife obesity and the risk for AD has been well described in epidemiological studies. Kivipelto and colleagues demonstrated in an follow-up study of 21 years that participants with midlife obesity had an increased risk for AD (Kivipelto et al., 2005). In a 27-year longitudinal population based study in 2005, Whitmer and colleagues demonstrated that midlife

obesity increased the risk for developing dementia by 74% (Whitmer et al., 2005b). Later the same group demonstrated in 2008 that central obesity at midlife increased the risk for dementia. By assessing the sagittal abdominal diameter as a measure of central obesity they found that an increase in sagittal abdominal diameter (> 25 cm) was correlated with increased risk for dementia (Whitmer et al., 2008). Fitzpatrick demonstrated in a prospective study of 2798 individuals without dementia that over a 5.4 year follow up period the probability of dementia was greater in obese adults at midlife (Fitzpatrick et al., 2009). Later a study from the Swedish Twin Registry, using 3,534 individuals showed that from 350 subjects diagnosed with dementia 29.8% of the cases had midlife obesity (Xu et al., 2011). More recent a follow-up of 26 years study showed that high BMI at midlife was associated with increased risk of incident of dementia, independently of obesity-related risk factors and co-morbidities (Tolppanen et al., 2014). Finally, a recent meta-analysis study to evaluate the risk factors for AD assessed 291 epidemiological studies and demonstrated that there was a 89% consistency of association between obesity and dementia (Deckers et al., 2015).

1.3.4.1 Experimental high-fat diet studies and Alzheimer's disease

In addition to clinical studies, experimental studies in rodents have also demonstrated that obesity and diet-induced obesity, through high-fat feeding, are associated with AD. Since the majority of obesity is due to an over consumption of diets that are rich in SFAs and cholesterol, such as Western diets, most experimental studies use diets to achieve an obese phenotype.

Recent studies have shown that high-fat and high-cholesterol diets can increase APP, Aβ and tau in mice (Thirumangalakudi et al., 2008, Jeon et al., 2012, Puig et al., 2012). This is consistent with epidemiological studies that demonstrate that in morbidly obese individuals without cognitive impairment, hallmarks of AD disorder, such as Aβ and tau expression, were present in the hippocampus (Nguyen et al., 2014). In addition, several studies have shown that the levels of plasma Aβ are increased in obese patients (Lee et al., 2009, Jahangiri et al., 2013) which correlates with increased body fat (Balakrishnan et al., 2005, Lee et al., 2009).

High-fat diets can also increase Aβ production, including Aβ oligomers and plaque formation in the brain of several AD mouse models (Shie et al., 2002, Ho et al., 2004, Oksman et al., 2006,

Julien et al., 2010, Kohjima et al., 2010, Barron et al., 2013, Vandal et al., 2014). Moreover, high-fat feeding can lead to insulin resistance (Ho et al., 2004, Kohjima et al., 2010, Mody et al., 2011, Barron et al., 2013), increased glucose intolerance (Mody et al., 2011, Barron et al., 2013, Vandal et al., 2014) and increased oxidative stress (Studzinski et al., 2009). Whilst in a high-fat diet has shown to not only affect Aβ metabolism but also increase AICD levels (George et al., 2004), other studies have demonstrated no change in Aβ or tau (Studzinski et al., 2009, Knight et al., 2014). Nevertheless, the majority of studies report that a high-fat diet exacerbates cognitive deficits in AD models and affects hippocampal-dependent memory (Refolo et al., 2000, Levin-Allerhand et al., 2002, Ho et al., 2004, Oksman et al., 2006, Julien et al., 2010, Maesako et al., 2012, Barron et al., 2013, Leboucher et al., 2013, Knight et al., 2014, Vandal et al., 2014). In particular in the 3xTgAD mice, a high-fat diet increases body weight, induces hyperglycemia and hyperinsulinemia, increases levels of soluble Aβ in the hippocampus as well levels of Aβ40 and Aβ42 in cortex, and reduces levels of postsynaptic marker debrin (Julien et al., 2010, Barron et al., 2013, Knight et al., 2014, Vandal et al., 2014). In all these studies a high-fat diet worsens the memory deficits (Barron et al., 2013, Knight et al., 2014, Vandal et al., 2014).

High-fat feeding also leads to memory and learning impairment in cognitively normal rodents (Molteni et al., 2002, Winocur and Greenwood, 2005, Jurdak et al., 2008, Stranahan et al., 2008, Jurdak and Kanarek, 2009, Pistell et al., 2010, Yu et al., 2010, Valladolid-Acebes et al., 2011, Kosari et al., 2012, Valladolid-Acebes et al., 2013, Arnold et al., 2014, Sobesky et al., 2014, Reichelt et al., 2015). In particular, high-fat feeding in rodents impairs hippocampal LTP in the dentate gyrus (Karimi et al., 2013, Reichelt et al., 2015) and CA1 regions (Stranahan et al., 2008), reduced synaptic plasticity (Molteni et al., 2002, Stranahan et al., 2008, Lynch et al., 2013, Reichelt et al., 2015) and synaptic proteins expression, such as postsynaptic density protein 95 (PSD95) (Arnold et al., 2014). Moreover, increased hippocampal insulin resistance (Arnold et al., 2014) and inflammation (Pistell et al., 2010, Sharma et al., 2014, Sobesky et al., 2014) together with reduced brain-derived neurotrophic factor (BDNF) (Molteni et al., 2002, Pistell et al., 2010, Sharma et al., 2014), increased astrocyte reactivity (Pistell et al., 2010) and oxidative stress (Ma et al., 2014, Sharma et al., 2014, Haohao et al., 2015) were also observed.

Overall, these data suggest that obesity, and in particular high-fat diets, led to changes in hippocampal structure that are associated with changes in cognition and are able to exacerbate AD pathology and memory deficits. These suggest that obesity and AD may share similar pathways to what concern cognition, however the underlying mechanism remains unknown.

1.4 The blood-brain barrier

In both AD and obesity studies have shown that BBB integrity is affected. The BBB is an effective and important barrier that allows the brain to be isolated from the periphery. Located between the blood and the cerebral tissue, the BBB is formed by an extensive network of capillaries composed by highly specialised endothelial cells that exist in the brain microvasculature (Wolburg and Lippoldt, 2002, Bourasset et al., 2009). The presence of tight junctions (TJs) between the endothelial cells, with no fenestration, and presence of specific transporters makes the endothelium a type of cells unique (Kim et al., 2005) that allows a well-controlled exchange between the brain and the blood. The function of the brain cerebrovasculature also depends on the interaction between other types of cells that together with TJs constitute the neurovascular unit (NVU) of the BBB, such as the endothelial cells, the basement membrane, astrocyte endfeet, pericytes, neurones and microglia (Persidsky et al., 2006, Choi and Kim, 2008) (Figure 4). Cerebral endothelial cells are also distinctive from peripheral vessels. Apart from the presence of TJs, cerebral endothelial cells are characterised by reduced endothelial vesicles or caveole, and increased number of mitochondria that provide the metabolic energy for maintaining the ionic gradient across the BBB (Nag, 2003). Overall the NVU controls BBB permeability and is involved in cerebral blood flow (CBF) regulation, immune surveillance, trophic support and homeostatic balance (Iadecola, 2010). As BBB function is essential for a normal and healthy central nervous system (CNS), BBB integrity compromise can impair neuronal function. Moreover, it is known that BBB integrity is compromised in inflammation and diseases, such as obesity and AD (Kanoski et al., 2010, Biron et al., 2011, Freeman and Granholm, 2012, Li et al., 2013, Lynch et al., 2013, Pepping et al., 2013, Hsu and Kanoski, 2014, Takeda et al., 2014, Tucsek et al., 2014b).

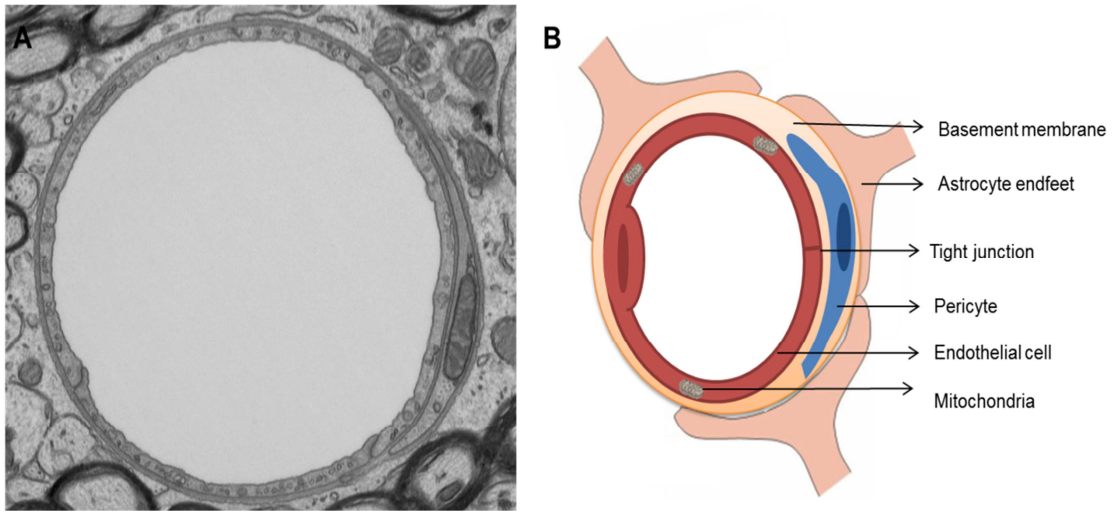

Figure 4. The blood-brain barrier. A) A electron micrograph of the BBB. **B)** A schematic representation of the BBB and its components.

1.4.1 Blood-brain barrier impairment and morphological changes in Alzheimer's disease and obesity

AD is associated with impairment of BBB function (Biron et al., 2011, Takeda et al., 2014, Li et al., 2015) and cerebrovascular endothelial dysfunction (Iadecola et al., 1999, Niwa et al., 2000, Paul et al., 2007, Zlokovic, 2011). Reduced and impaired microvascular density with increased number of fragmented vessels (Farkas and Luiten, 2001, Bailey et al., 2004) marked changes in vessel diameter (Bailey et al., 2004), thickening of the basement membrane (Kalaria, 1996, Grammas et al., 2002) and reduced endothelial mitochondria number (Marlatt et al., 2008) are observed in the brain of AD patients. At a biochemical level at the cerebrovasculature, AD patients present increased levels of pro-inflammatory cytokines, such as TNF-α and IL-6 that are released from the vessels (Grammas, 2011), endothelial inflammation, characterised by increased levels of vascular cell adhesion molecule 1 (VCAM-1) (Zuliani et al., 2008) and intercellular adhesion molecule 1 (ICAM-1) (Grammas, 2011). AD patients also have a defective glucose transport at the BBB (Banks, 2010).

Aβ is thought to play an important role in BBB integrity. Indeed, it is in the BBB that the two receptors that mediate Aβ transport are present: LRP, responsible for the efflux of Aβ (clearance) (Zlokovic et al., 2010), and receptor for advanced glycation end products (RAGE), that is involved in the influx of Aβ into the brain (Yan et al., 1996). Several studies from AD patients and animals have demonstrated that AD leads to an increase in RAGE expression and/or a decrease in LRP expression in the BBB, which consequently leads to Aβ accumulation (Deane et al., 2004, Bell and Zlokovic, 2009). In addition, Aβ efflux is decreased in both transgenic mice and in the SAMP8 mouse, a model with a natural mutation that leads to aging, increased Aβ production and memory deficits (Banks et al., 2003, LaRue et al., 2004, Kandimalla et al., 2005, Banks, 2010).

In transgenic AD mice several studies demonstrate that the BBB is impaired, evaluated by the presence of certain dyes in the brain parenchyma of these mice (Ujiie et al., 2003, Dickstein et al., 2006, Hohsfield et al., 2014), and by the disruption of TJs proteins (Biron et al., 2011). In 3xTgAD mice an increase basement membrane thickness at 11 months, a reduction in vascular volume of the hippocampus at 18 months and a deficiency in BBB glucose transporters (Do et al., 2014) between 6 and 9-months has been observed (Ding et al., 2013).

As a risk factor for AD, obesity can also affect BBB function. Although less is known in obese patients, diet-induced obesity studies in rodents, through high-fat feeding, has demonstrated that BBB permeability is increased, which could contribute to vascular and BBB dysfunction (Kanoski et al., 2010, Davidson et al., 2012, Lynch et al., 2013, Chang et al., 2014, Ouyang et al., 2014). Interestingly, the brain region that appear to be more vulnerable to a high-fat diet is the hippocampus, where reduced BBB integrity and increase microgliosis has been observed (Freeman and Granholm, 2012). Moreover, evidence suggests that high circulating levels of fat can impair the BBB transport of important hormones, such as leptin, ghrelin (Banks et al., 2004, Banks et al., 2008) and insulin (Heni et al., 2014, Spielman et al., 2014) which could contribute to other characteristics seen in obesity, such as insulin resistance.

Taken together, these data demonstrates that both AD and obesity can induce BBB dysfunction and more importantly hippocampal damage. However, how exactly AD and obesity affect BBB and how BBB dysfunction can affect memory remains unknown.

1.5 Objectives

Obesity is known as a risk factor for developing AD later in life. Furthermore, experimental high-fat diets have demonstrated to impair memory in cognitively normal mice and also in AD mouse models. However, the underlying detrimental effects of obesity and high-fat diets on cognition are unknown.

AD prevalence is affected by gender, as sex being one of the risk factors for the disease, with women more susceptible to develop AD than man. In addition, obesity is also associated with gender differences, as distribution of WAT differs between sexes and in experimental high-fat diet studies female rodents are known to have different metabolic responses to diet than males. This suggests that gender could influence the effect of AD and obesity on cognitive impairment and other molecular events associated with both diseases.

Memory impairment is a common feature of both obesity and AD and is associated with several other molecular events that are involved in both disorders, such as inflammation, T2D, insulin resistance and BBB impairment. Moreover, the particular role of each molecular phenomenon on cognition, as well the molecular mechanisms behind the effect of a high-fat diet on memory remains unclear. Therefore, the overall aim of this PhD is to further understand the role of gender on the impact of a high-fat diet on memory in both cognitively normal and cognitively impaired mice and try to understand possible molecular mechanisms for its effect. Therefore the main aims were to:

- evaluate the role of a high-fat diet on both neuropathology (Aβ oligomers) and neuroinflammation (microglia) in male 3xTgAD mice;
- compare the effect of a high-fat diet on memory in male and female Non-Tg and 3xTgAD mice;
- evaluate if memory deficits induced by a high-fat diet and in AD are associated with changes in BBB structure, mitochondria and synapses in male and female Non-Tg and 3xTgAD mice;
- evaluate if the detrimental effects of a high-fat diet are dependent on obesity.

Chapter 2

High-fat diet-induced memory impairment in triple transgenic Alzheimer's disease (3xTgAD) mice is independent of changes in amyloid and tau pathology

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Neurobiology of Aging, 2014

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Declaration

This paper includes material submitted to Elysse Knight PhD thesis and therefore will not be discussed in this thesis. The only work that will be discuss correspond to Aβ oligomer ELISA and microglia data that has been performed by Isaura Martins.

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High-fat diet-induced memory impairment in triple-transgenic Alzheimer's disease (3xTgAD) mice is independent of changes in amyloid and tau pathology☆

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ABSTRACT

Obesity and consumption of a high-fat diet are known to increase the risk of Alzheimer's disease (AD). Diets high in fat also increase disease neuropathology and/or cognitive deficits in AD mouse models. However, the effect of a high-fat diet on both the neuropathology and memory impairments in the triple-transgenic mouse model of AD (3xTgAD) is unknown. Therefore, groups of 2-month-old male 3xTgAD and control (non-Tg) mice were maintained on a high-fat or control diet and memory was assessed at the age of 3–4, 7–8, 11–12, and 15–16 months using a series of behavioral tests. A comparable increase in body weight was observed in non-Tg and 3xTgAD mice after high-fat feeding at all ages tested but a significantly greater increase in epididymal adipose tissue was observed in 3xTgAD mice at the age of 7–8, 11–12, and 15–16 months. A high-fat diet caused memory impairments in non-Tg control mice as early as the age of 3–4 months. In 3xTgAD mice, high-fat consumption led to a reduction in the age of onset and an increase in the extent of memory impairments. Some of these effects of high-fat diet on cognition in non-Tg and 3xTgAD mice were transient, and the age at which cognitive impairment was detected depended on the behavioral test. The effect of high-fat diet on memory in the 3xTgAD mice was independent of changes in AD neuropathology as no significant differences in (plaques, oligomers) or tau neuropathology were observed. An acute increase in microglial activation was seen in high-fat fed 3xTgAD mice at the age of 3–4 months but in non-Tg control mice microglial activation was not observed until the age of 15–16 months. These data indicate therefore that a high-fat diet has rapid and long-lasting negative effects on memory in both control and AD mice that are associated with neuroinflammation, but independent of changes in beta amyloid and tau neuropathology in the AD mice.

1. Introduction

Alzheimer's disease (AD) is the most common form of dementia and is a significant health problem worldwide. AD is characterized by the presence of beta amyloid (Aβ) plaques and neurofibrillary tangles within the brain and patients present with cognitive deficits including impairments in learning and memory. The occurrence of AD is mostly sporadic affecting individuals over the age of 65 years. However, there are several factors that can increase AD risk including diabetes, stroke, atherosclerosis, and obesity and/or metabolic syndrome.

Obesity is a major health problem associated with increased risk of several diseases such as diabetes. However, obesity at midlife can also increase the risk of dementia and AD later in life (Beydoun et al., 2008; Fitzpatrick et al., 2009; Gustafson et al., 2003; Hassing et al., 2009; Kivipelto et al., 2005; Profenno et al., 2009; Rosengren et al., 2005; Whitmer et al., 2005, 2007, 2008), an effect that is independent of the conditions associated with obesity that are also risk factors for AD, such as type 2 diabetes and cardiovascular disease (Hassing et al., 2009; Whitmer et al., 2005, 2007). This relationship between obesity and AD appears to depend on age as obesity can decrease the risk of AD in later life (Fitzpatrick et al., 2009) and weight loss actually precedes disease onset (Buchman et al., 2005; Stewart et al., 2005). Obesity is often caused by and is associated with, consumption of diets that are high in fat. The prevalence of AD is greater in countries with higher intake of high fat and/or calorie diets but lower in those that consume diets low in fat (Grant, 1997; Panza et al., 2004). Furthermore, epidemiologic studies suggest diets high in

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saturated fats (especially in midlife) are a major risk factor for the development of AD (Eskelinen et al., 2008; Grant, 1999; Kalmijn et al., 1997; Laitinen et al., 2006; Luchsinger et al., 2002), and this risk is higher in individuals with the APOE ε4 allele (Laitinen et al., 2006; Luchsinger et al., 2002).

Disease neuropathology and/or behavioral deficits are enhanced in mouse models of AD that are maintained on a high-fat diet (without high levels of cholesterol) (Herculano et al., 2013; Ho et al., 2004; Julien et al., 2010; Maesako et al., 2012a; Pedrini et al., 2009; Phivilay et al., 2009). There are several well-characterized mouse models of AD, most with mutations in amyloid precursor protein (APP) and/or presenilin 1/2 (PS1/2) that present with Aβ plaques only. The triple-transgenic AD (3xTgAD) mouse has mutations in APP_{Swe}, $PS1_{M146V}$, and tau_{P301L}, and as a consequence, develops temporal- and region-specific Aβ plaques and tangle-like pathology that closely resemble the pathology seen in the human AD brain, in addition to developing functional impairments, including learning and memory deficits (Billings et al., 2005; Oddo et al., 2003a, 2003b). The 3xTgAD mouse does not present with an aggressive pathology, as we do not observe Aβ plaques and tangle-like pathology until approximately at the age of 12 months, which is after cognitive deficits are detected (Billings et al., 2005; Knight et al., 2012, 2013). The 3xTgAD mouse therefore allows us to identify the effect of a high-fat diet before significant AD neuropathology, and to also study the relationship between Aβ plaques and tau.

In humans, the severity of AD-related neuropathology especially Aβ plaque burden, does not always correlate with, or is predictive of, cognitive deficits, and memory impairments can occur in mouse AD models in advance of overt Aβ plaque (and tangle) pathology (Billings et al., 2005; Oddo et al., 2003b; Serrano-Pozo et al., 2011). Most studies to date examining the role of high-fat diets in AD mouse models have assessed neuropathology only (Julien et al., 2010; Pedrini et al., 2009; Phivilay et al., 2009) and few have monitored both neuropathology and memory (Herculano et al., 2013; Ho et al., 2004; Maesako et al., 2012a). Furthermore, most of these studies identifying an effect of a high-fat diet in AD on neuropathology and behavior have modified diet in AD mice only and have not studied the effect of diet in control animals (Ho et al., 2004; Maesako et al., 2012a). As high-fat diets have been shown to affect memory in cognitively-normal rodents (McNeilly et al., 2011; Pistell et al., 2010; Winocur and Greenwood, 2005), it is not clear whether the cognitive deficits observed in AD mice fed a high-fat diet are related to or independent of AD pathology. Finally, most reports on the changes in cognition and/or neuropathology in high-fat fed AD mice have studied just one time point, and thus only report effects at a single stage and/or severity of the disease. It is possible that some of the effects of a high-fat diet in AD might be transient, which will be missed in such studies.

The aim of this study therefore was to characterize longitudinally the impact of a high-fat diet on both cognition and neuropathology in male 3xTgAD and non-transgenic (non-Tg) control mice. Memory was assessed using a battery of behavioral tests. No study to date has compared the effects of a high-fat diet on both cognition and neuropathology in 3xTgAD mice and we show that a high-fat diet impairs memory in both the non-Tg control and 3xTgAD mice, the effects of which depend on the behavioral test used and duration of diet. Effects of high-fat diet on cognition in the 3xTgAD mice occurred without any significant effect on AD neuropathology.

2. Methods

2.1. Animals and diet

Male 3xTgAD mice expressing mutant $PS1_{M146V}$, APP_{Swe}, Tau_{P301L}, and control non-Tg (129/C57BL6) mice were originally supplied by Frank LaFerla (Irvine, CA, USA) (Oddo et al., 2003b) and an in-house colony established in Manchester. All mice were kept in standard housing conditions (humidity 50%–60%, temperature 21 ± 1 °C, 12:12 hour light-dark cycle with lights on at 07:00 hours) and given *ad libitum* access to standard rodent chow and water unless stated. All animal experiments were carried out in accordance with the United Kingdom Animals (Scientific Procedures) Act 1986. At the age of 8 weeks 3xTgAD and non-Tg control mice were placed on either a high-fat diet (60% energy from fat, 35% fat content by weight, 13% saturated fatty acids, 58G9, Test Diets, supplied by IPS Product Supplies Ltd, UK) or control diet (12% energy from fat, 5% fat content by weight, 0.78% saturated fatty acids, 58G7). Separate groups of mice were maintained on their respective diets until the age of 3–4 (n = 10–12/group), 7–8 (n = 10–11/group), 11–12 (n = 9–10/group), or 15–16 (n = 6–10/group) months when behavioral tests were performed. Body weight was monitored in all mice from weaning until behavioral assessment. There were a few deaths because of unknown causes over the cause of the study, and these animals were not included in the analyses (7–8 months: non-Tg control n = 1; 11–12 months: 3xTgAD control n = 1, 3xTgAD high-fat n = 1; 15–16 months: non-Tg high-fat n = 3, 3xTgAD control n = 2, 3xTgAD high-fat n = 1).

2.2. Behavioral tests

Male non-Tg control and 3xTgAD mice were subjected to the Y-maze spontaneous alternation, smell recognition, novel object recognition, and Morris water maze (MWM) tests. On the days of behavioral evaluation, home cages were placed in the testing room 30 minutes before testing to allow habituation. All behavioral observations were made between 1000 hours and 1600 hours. The order of observation during this period was randomized across animals and all subsequent analysis was performed blinded to genotype and diet. No more than one behavioral test was completed during any single day. All equipment was cleaned between animals.

2.2.1. Y-maze spontaneous alternation test

Short-term working memory was assessed in the Y-maze spontaneous alternation test using a black opaque Perspex Y-maze with 3 arms (A, B, and C) each containing a visual cue (arm dimensions; 15 cm × 10 cm × 10 cm). Each animal was placed in turn in arm A of the Y-maze and allowed to explore for 8 minutes and the arm entries made by each animal were recorded. Arm entry was defined as having all 4 paws in the arm. Spontaneous alternation was defined as a successive entry into 3 different arms, on overlapping triplet sets (Hiramatsu et al., 1997; Wall and Messier, 2002). The percentage number of alternations was calculated as the number of actual alternations divided by the maximum number of alternations (the total number of arm entries minus 2). The total number of moves was also recorded as an index of ambulatory activity (Hiramatsu et al., 1997).

2.2.2. Smell recognition test

Short-term non-associative memory based on the natural exploration of novelty in mice was assessed in the smell recognition test. All mice were habituated to a black opaque polycarbonate circular arena (diameter, 30 cm × height, 21 cm) for 5 minutes over 2 days. On the day of testing, mice were placed in the arena and allowed to explore 2 identical unfamiliar scented balls for 10 minutes (phase 1). The scented balls were placed in the center of the arena, 5 cm from the edge and 8 cm away from each other. The hollow balls (Chad Valley, UK) were filled with cotton wool and 0.5 mL of scent (orange, lemon, vanilla, or almond, Dr Oetker Ltd, UK) was evenly distributed into the balls via small holes. Mice were then removed, one of the balls was replaced with a novel scented

ball, and after a delay of 3 minutes, mice were placed back into the arena and allowed to explore for a further 4 minutes (phase 2). The novel scented ball was placed randomly in either the left or right position. All behavior was recorded with a camera (Sanyo Xacti VPC-C4, SANYO Fisher, CA, USA) and MP4 video-clips were converted to an AVI format using Pazera MP4 to AVI converter 1.3 (Pazera-Software, PL). The duration (seconds) spent exploring the scented balls was then measured using Observer 5.0 software (Noldus, Wageningen, the Netherlands). Exploration was defined as the amount of time the animals spent with their nose pointing within 2 cm of the scented balls. The percentage time spent exploring the scented balls was calculated for phases 1 and 2.

2.2.3. Novel object recognition test

Short-term non-associative memory based on the natural exploration of novelty in mice was also assessed in the novel object recognition test. The task was performed the same as the smell recognition task, but during phase 1 of the task the mice were placed in the arena and allowed to explore 2 identical unfamiliar wooden painted trial objects (Universe of Imagination, Geoffrey, Inc, UK) for 10 minutes. Mice were then removed and one of the objects was replaced with a novel object that varied in shape and color. After an interval of 1 hour, mice were allowed to explore the familiar object and the novel object for 4 minutes. The time spent exploring the familiar and novel objects was calculated for phases 1 and 2 as for the smell recognition test.

2.2.4. MWM

Spatial reference memory was assessed using the MWM using a 1.2 m diameter (height, 25 cm) circular white opaque plastic tank that contained water maintained at a temperature of 21 °C–22 °C and made opaque using water-soluble nontoxic white paint (Universe of Imagination, Geoffrey Inc, UK). During the MWM test, mice were given 2 days of visual platform training followed by 8 days of hidden platform training and a 1 day probe trial (based on protocol by Vorhees and Williams (2006)). Briefly, for the acquisition of the visual platform training, mice were placed into the maze without spatial cues, and allowed to locate a visual flagged platform. If the platform was not found within 2 minutes, the mouse was gently guided to it. Mice were given 4 trials each day for 2 days with a different start position and flagged platform location each trial. For the acquisition of the hidden platform test, 4 trials per day were conducted for 8 days. The sequence of start positions was different on each training day and visual spatial cues were located outside the tank. The latency to find the platform was recorded with a maximum of 2 minutes allowed. To test memory retention of the platform location, mice underwent a probe trial 24 hours after the final hidden platform training trial. During the probe trial, the platform was removed, and the mouse was placed in the pool and allowed to swim for 30 seconds. Time spent in each quadrant was recorded. Each trial was monitored and analyzed using a CCTV tracking camera (Vista protos IV, UK) and 2020 PLUS tracking software (HVS Image, Buckingham, UK). The escape latency (second) during both visual platform and hidden platform training and the percentage time in the target quadrant during the probe trial and swim speed (meter/second) were calculated.

2.3. Tissue preparation

After the behavioral tests all animals were terminally anesthetized with 3.5% isoflurane (30% O_2 and 70% N_2O) and 0.9% saline was perfused-transcardially. The brain was rapidly removed and one hemisphere immerse-fixed in 4% paraformaldehyde at room temperature for 24 hours. This hemisphere was then cryoprotected in 30% sucrose (in 0.1 M phosphate buffer [PB]) at 4 °C for 24 hours before being frozen in isopentane on dry ice. The hippocampus was dissected from the other hemisphere of the brain and frozen on dry ice. All samples were stored at −80 °C until assay. One epididymal fat pad was also dissected and weighed.

2.3.1. Immunohistochemistry

Coronal 30 μm brain sections were cut (from −1.34 mm to −3.88 mm relative to bregma according to the atlas of Paxinos and Franklin (2001)), on a freezing sliding microtome (Bright 8000-001, Bright Instrument Co Ltd, UK). Immunohistochemistry for either Aβ or phosphorylated tau was then performed on free-floating brain sections. Briefly, endogenous peroxidase was removed before treatment in blocking solution (10% normal horse serum in PB/0.3% triton). Sections were then incubated at 4 °C overnight with either a mouse monoclonal anti-human amyloid 6E10 (1:3000, Covance-Signet Laboratories, UK) for Aβ or mouse monoclonal anti-human PHF-tau (AT8; 1:1000, Autogen Bioclear, UK) for hyperphosphorylated tau. After washes in PB/0.3% triton, sections were treated for 2 hours in a biotinylated horse anti-mouse IgG antibody (1:500; Vector Laboratories Ltd, Peterborough, UK). Following washes (in 0.1 M PB), sections were immersed in avidin-biotin-peroxidase complex (ABC, Vector Laboratories Ltd) for 30 minutes, rinsed in 0.1 M PB and color-developed using a 0.05% diaminobenzidine solution (in 0.01% H_2O_2). Sections were mounted onto gelatin-coated slides, dried, and coverslipped before viewing under a light microscope. The number of immunopositive cells (neurons) expressing tau was counted unilaterally, using a light microscope, throughout the hippocampus. The average number of cells per section was calculated and the group mean determined. The Aβ plaque burden was determined throughout in the hippocampus. The plaque area was measured in each section and the plaque burden calculated as average plaque area/section (μm^2).

To detect microglia, immunohistochemistry was performed as previously mentioned but using a rabbit anti-Iba1 primary antibody (1:2000, Wako Chemicals, Germany) and a biotinylated goat anti-rabbit IgG antibody (1:500; Vector Laboratories Ltd), all in 2% normal goat serum in PB/0.3% triton. Activated microglia throughout the hippocampus were identified according to previous studies. Drake et al., 2011 defined by either an increase in Iba1 staining; enlarged cell bodies; complete or partial loss of thin elongated process. The number of activated microglia per section was counted and the average calculated. For all immunohistochemical analyses the investigator was blinded to genotype and diet.

2.3.2. Aβ oligomer enzyme-linked immunosorbent assay

The hippocampus of control and high-fat fed 3xTgAD mice, at all ages tested, were prepared by homogenization in extraction buffer (50 mM Tris-Cl, 150 mM NaCl, 1% CHAPS, pH 7.6, containing proteases inhibitors) and samples were left to stand for 3 hours. The homogenates were then centrifuged at 15,000 rpm at 4 °C for 30 minutes. Human Aβ oligomers were analyzed in the hippocampal supernatant by enzyme-linked immunosorbent assay (ELISA) (IBL International, Germany) according to manufacturer's instructions. The ELISA uses mouse monoclonal anti-human Aβ (N) (82E1) antibodies that recognize the N-terminus of human Aβ specifically, with 2 or more epitopes. The level of oligomers in the hippocampus of non-Tg control mice was not tested, as no human oligomers are detected in the brain of these mice (data not shown).

2.4. Statistical analysis

Data are represented as mean ± standard error of the mean. Epididymal fat weight, evaluation of memory in the spontaneous

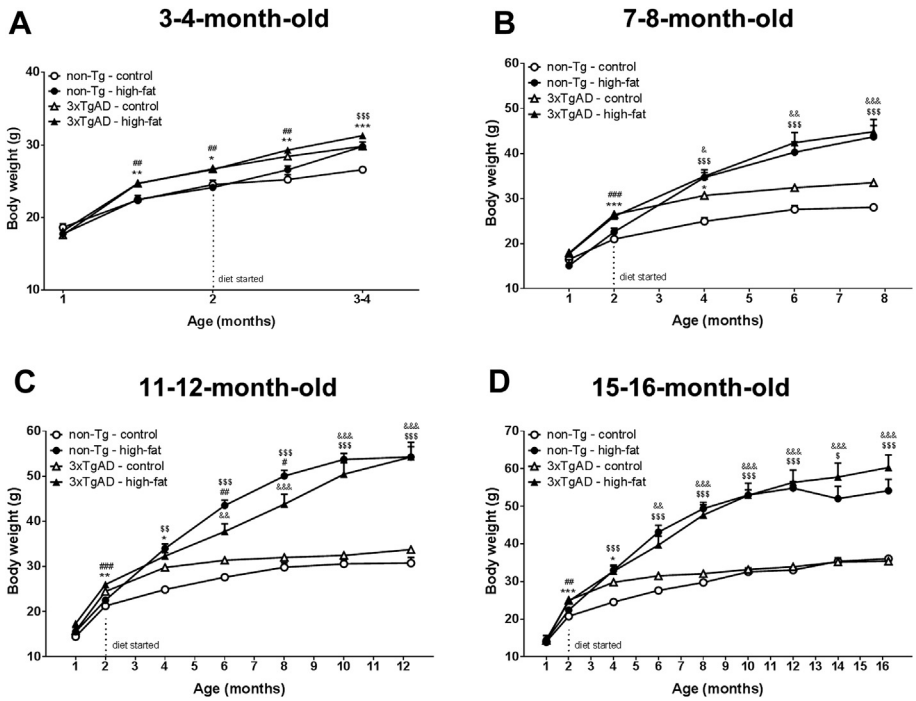

Fig. 1. Body weight of 3xTgAD and non-Tg mice in response to a high-fat diet. Male 3xTgAD and non-Tg control mice were maintained on either a control or high-fat diet from the age of 8 weeks (at the age of 2 months) and body weight was assessed in separate groups of mice at the age of 3–4 (A), 7–8 (B), 11–12 (C), and 15–16 (D) months. Data are means ± SEM, n = 6–12/group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ non-Tg versus 3xTgAD mice on control diet; ## $p < 0.01$, ### $p < 0.001$ non-Tg versus 3xTgAD mice on high-fat diet; $ $p < 0.05$, $$ $p < 0.01$, $$$ $p < 0.001$ non-Tg on a control versus high-fat diet; & $p < 0.05$, && $p < 0.01$, &&& $p < 0.001$ 3xTgAD on a control versus high-fat diet. Three-way repeated measures ANOVAs with Scheffe post hoc analysis. Abbreviations: ANOVA, analysis of variance; SEM, standard error of the mean.

alternation test, and the number of activated microglia were examined using a 2-way analysis of variance (ANOVA) with Bonferroni post hoc analysis and for the smell recognition, novel object tests, and neuropathology a Student *t* tests was used. Morris water maze training was assessed between cohorts on individual training days and compared within cohorts between the first day of training and successive days of training to assess improvement over time via 3-way repeated measures ANOVAs with Scheffe post hoc analysis, as was body weight. The probe test was analyzed using a 2-way ANOVA with Bonferroni post hoc analysis. Statistical significance was taken at $p < 0.05$.

3. Results

3.1. High-fat diet increased body weight and fat mass in both non-Tg and 3xTgAD mice

For all groups of mice at the age of 1 month, there was no significant difference in body weight between non-Tg and 3xTgAD mice (Fig. 1). By the age 2 months, before modifying diet, 3xTgAD mice weighed significantly ($p < 0.05$–0.001) more than non-Tg control mice in all groups.

At the age of 3–4 months, non-Tg mice on a high-fat diet were significantly ($p < 0.001$; Fig. 1A) heavier than those maintained on a control diet, but no difference was observed between 3xTgAD mice on a control versus high-fat diet. As at the age of 2 months, 3 to 4-month-old 3xTgAD mice weighed significantly ($p < 0.001$) more than non-Tg mice when on a control diet. At the age of 7–8, 11–12, and 15–16 months, both 3xTgAD and non-Tg mice on a high-fat diet weighed more than their respective control fed mice, and there was now no difference in body weight between control-fed non-Tg and 3xTgAD mice (Fig. 1B–D).

At all ages tested male 3xTgAD mice on a control diet displayed no difference in epididymal fat pad weight compared with the control fed non-Tg mice (Table 1). High-fat feeding led to a significant ($p < 0.001$) increase in epididymal fat pad weight in both 3xTgAD and non-Tg mice at the age of 3–4 and 7–8 months compared with their respective control fed mice. Epididymal fat pad weight was significantly ($p < 0.01$–0.001) lower in the 3xTgAD than the non-Tg mice on a high-fat diet at the age of 3–4 months but higher in 3xTgAD mice at the age of 7–8 months. At the age of 11–12 and 15–16 months, epididymal fat pad weight was not different in non-Tg mice on a high-fat diet, whereas it was significantly ($p < 0.001$) higher in the 3xTgAD mice on a high-fat diet compared with their respective control fed mice.

3.2. High-fat diet impaired memory in non-Tg and 3xTgAD mice

3.2.1. Y-maze

At the age of 3–4 months, there was no significant effect of genotype or diet on memory performance in the Y-maze (Fig. 2A). By the age of 7–8 months, 3xTgAD mice showed a diet-independent decrease (18% and 24%; $p < 0.05$) in percentage

Table 1
Epididymal fat weight of 3xTgAD and non-Tg mice in response to a high-fat diet

	Non-Tg		3xTgAD	
	Control	High-fat	Control	High-fat
3–4 mo	0.50 ± 0.04	1.46 ± 0.12[a]	0.37 ± 0.02	1.00 ± 0.09[a,c]
7–8 mo	0.55 ± 0.08	1.62 ± 0.09[a]	0.50 ± 0.04	2.51 ± 0.36[a,b]
11–12 mo	0.73 ± 0.11	1.50 ± 0.09	0.54 ± 0.05	4.75 ± 0.59[a,c]
15–16 mo	1.21 ± 0.14	1.35 ± 0.27	0.67 ± 0.09	5.56 ± 0.85[a,c]

Mice were maintained on a high-fat or control diet and separate groups of mice were monitored until the age of 3–4, 7–8, 11–12, or 15–16 months when epididymal fat pad weight (g) was assessed. Data are mean ± SEM, n = 6–12/group. [a] $p < 0.001$ versus control fed mice of same genotype and [b] $p < 0.01$, [c] $p < 0.001$ versus non-Tg mice on the same diet. Two-way ANOVA with Bonferroni post hoc analysis.
Key: ANOVA, analysis of variance; SEM, standard error of the mean.

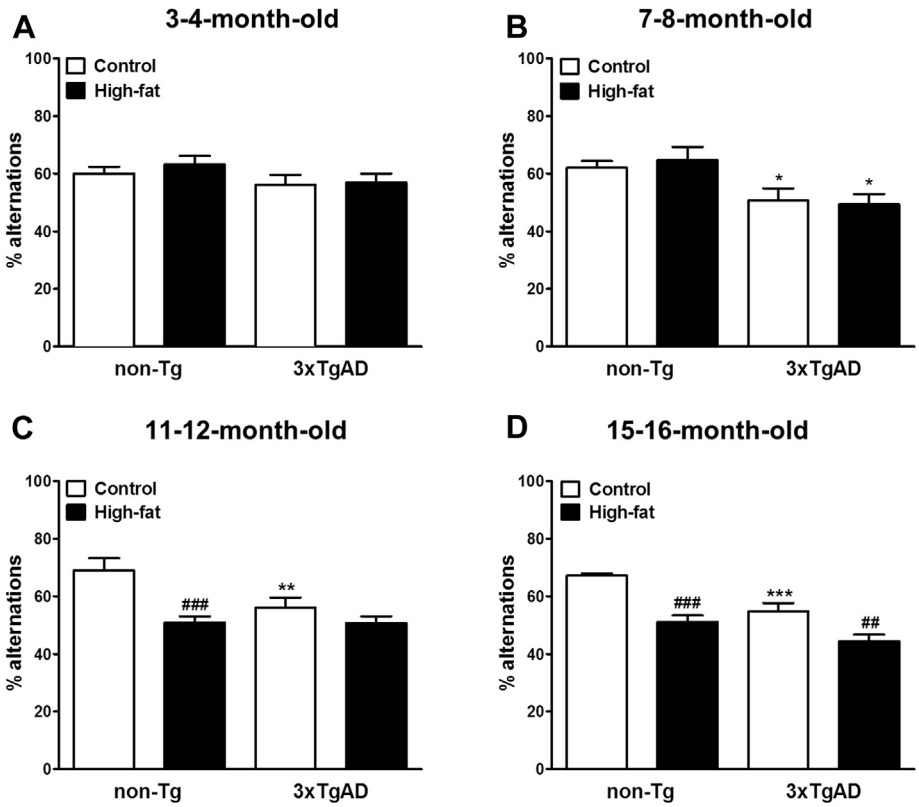

Fig. 2. High-fat diet impairs learning in the Y-maze spontaneous alternation test in 3xTgAD and non-Tg mice. Male 3xTgAD and non-Tg control mice were maintained on either a control or high-fat diet from the age of 8 weeks. Cognitive function was assessed in the Y-maze spontaneous alternation test in separate groups of mice at the age of 3–4 (A), 7–8 (B), 11–12 (C), and 15–16 (D) months. Data are means ± SEM. n = 6–12/group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ versus non-Tg mice on the same diet, ## $p < 0.01$, ### $p < 0.001$ versus control-fed mice of the same genotype. Two-way ANOVA with Bonferroni post hoc analysis. Abbreviations: ANOVA, analysis of variance; SEM, standard error of the mean.

alternations compared with non-Tg mice (Fig. 2B). Significantly, fewer percentage alternations (19%; $p < 0.01$) were still detected in 11 to 12-month-old control fed 3xTgAD compared with non-Tg mice (Fig. 2C). At the age of 11–12 months, high-fat diet impaired memory (26% reduction; $p < 0.001$) in non-Tg mice. However, a high-fat diet did not affect memory in 11 to 12-month-old 3xTgAD mice. At the age of 15–16 months, 3xTgAD mice on a control diet performed fewer percentage alternations (19% $p < 0.001$; Fig. 2D) than non-Tg mice. A high-fat diet not only impaired memory in 15 to 16-month-old non-Tg mice but also affected cognition in 3xTgAD mice as less percentage alternations were observed in high-fat compared with control fed mice for both genotypes (24% and 19%; $p < 0.001$ and $p < 0.01$ for non-Tg and 3xTgAD, respectively). The number of moves was transiently decreased in 3xTgAD mice at the age of 3–4 and 7–8 months but at all ages tested, a high-fat diet had no effect on the total number of moves in both non-Tg and 3xTgAD mice (data not shown).

3.2.2. Smell recognition

At the age of 3–4, 7–8, 11–12, and 15–16 months, there was no difference in exploration of identical scented balls during phase 1 of the smell recognition test for all groups of mice (data not shown). During phase 2, after a delay of 3 minutes, non-Tg mice at all ages on a control diet spent a significantly ($p < 0.05$) higher percentage of time exploring the novel scented ball than the familiar scented ball indicating memory formation (Fig. 3A–D). Memory was unimpaired in 3xTgAD mice on a control diet at earlier time points as mice spent more time exploring the novel scented ball at the age of 3–4 months ($p < 0.01$) and more time exploring the familiar scented ball at the age 7–8 months ($p < 0.05$). However, memory was impaired in control fed 3xTgAD mice at the age of 11–12 and 15–16 months as no difference in exploration between the novel and familiar smell was observed. High-fat feeding transiently impaired memory in non-Tg mice as no preference for exploring the novel or familiar scented ball was detected at the age of 3–4 and 7–8 months but by the age of 11–12 and 15–16 months high-fat fed non-Tg mice spent more time exploring the novel scented ball ($p < 0.05$). In contrast, the 3xTgAD mice on a high-fat diet showed impaired memory at all ages, as they did not show a preference for the novel or familiar smell.

3.2.3. Novel object recognition

At the age of 3–4, 7–8, 11–12, and 15–16 months, there was no difference in exploration of identical objects during phase 1 of the test in both non-Tg and 3xTgAD mice (data not shown). At the age of 3–4 and 7–8 months, during phase 2 after an interval of 1 hour, non-Tg mice on a control or high-fat diet spent significantly ($p < 0.05$ and $p < 0.001$) more time exploring the novel versus the familiar object (Fig. 4A and B). At the age of 11–12 and 15–16 months, the non-Tg mice on a control diet also spent significantly more time exploring the novel versus the familiar object ($p < 0.01$, Fig. 4C and D), whereas the high-fat diet impaired memory at these ages. In 3xTgAD mice, memory was impaired at all ages on both control and high-fat diet (Fig. 4A–D).

3.2.4. Morris water maze

By the second day of visual platform training in the MWM there was no significant difference in escape latency between any group at the age of 3–4, 7–8, 11–12, or 15–16 months (data not shown).

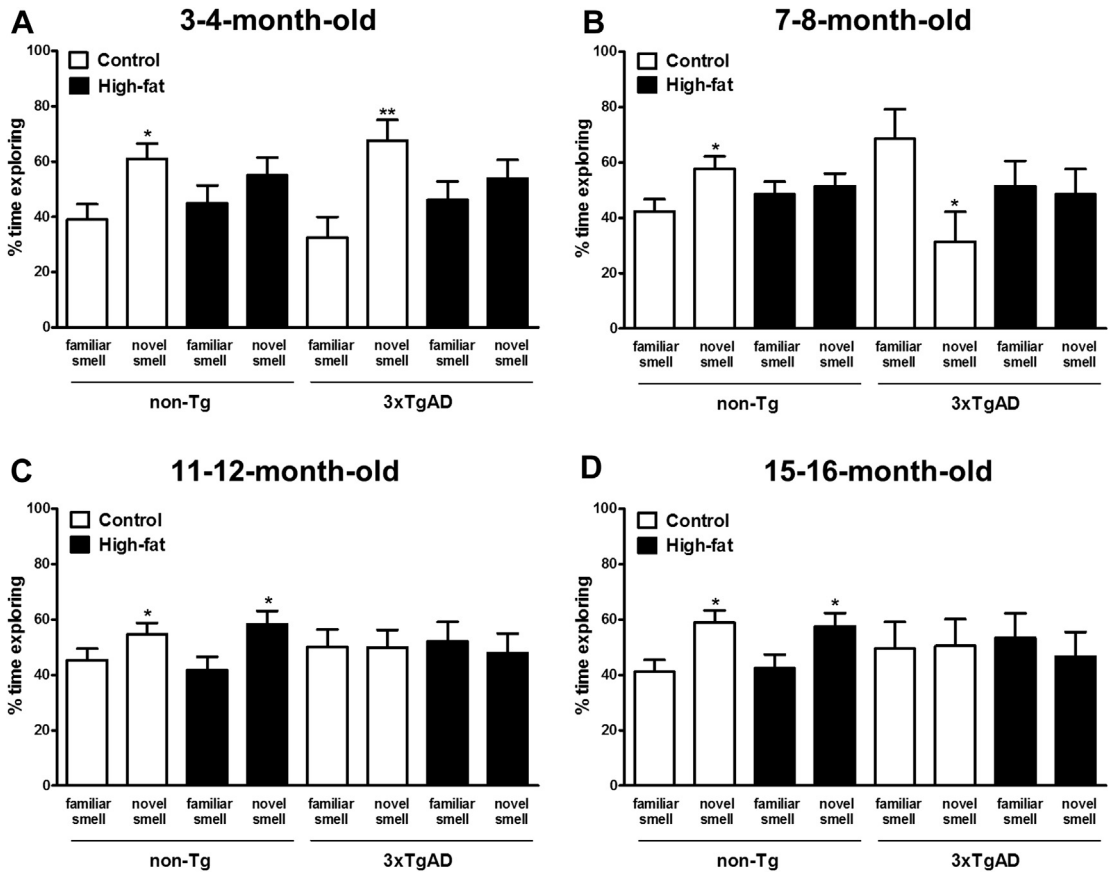

Fig. 3. High-fat diet impairs learning in the smell recognition test in 3xTgAD and non-Tg mice. Male 3xTgAD and non-Tg control mice were maintained on either a control or high-fat diet from the age of 8 weeks. Cognitive function was assessed in the smell recognition test in separate groups of mice at the age of 3–4 (A), 7–8 (B), 11–12 (C), and 15–16 (D) months. During phase 1, the mice were placed in an arena for 10 minutes with 2 identically scented balls (data not shown). During phase 2, after an interval of 3 minutes, the mice were placed back into the arena with one familiar scented ball (presented in phase 1) and one novel scented ball for 4 minutes and percentage time exploring balls was compared. Data are means ± SEM. n = 6–12/group. * $p < 0.05$, ** $p < 0.01$ for novel versus familiar smell; Student t test. Abbreviation: SEM, standard error of the mean.

During hidden platform training (Fig. 5A–D), non-Tg mice on a control diet showed significantly decreasing escape latency over training days at all ages tested (days 2–8 versus day 1; $p < 0.05$–0.001), whereas the 3xTgAD mice on a control diet showed no evidence of learning. On a high-fat diet, non-Tg mice got quicker over training days at the age of 3–4, 7–8, 11–12, and 15–16 months (days 2–8 versus day 1; $p < 0.05$–0.001), whereas the 3xTgAD mice on a high-fat diet, got quicker only at the age of 3–4 months (days 7–8 versus day 1; $p < 0.05$) yet were unable to learn at the age of 7–8, 11–12, and 15–16 months. On individual training days there was no significant difference in escape latency between the cohorts.

At all ages tested during the probe test, 3xTgAD mice on either a control or high-fat diet spent significantly less time in the target quadrant when compared with their respective fed non-Tg mice ($p < 0.05$–0.001, Fig. 5E–H). High-fat feeding had no effect on memory in 3 to 4-month-old 3xTgAD or non-Tg mice. However, by the age of 7–8 months, a high-fat diet impaired memory as a decrease (30%–32%) in the time spent in the target quadrant was observed in both high-fat fed 3xTgAD and non-Tg mice when compared with control fed mice. Effects of high-fat diet on memory were transient as at the age of 11–12 and 15–16 months no difference in time spent in the target quadrant was observed between 3xTgAD or non-Tg mice on either a control or high-fat diet. During the probe test at the age of 3–4 months swim speed was significantly higher in 3xTgAD mice compared with non-Tg control mice on either diet (non-Tg, control 0.23 ± 0.01 m/s, high-fat 0.24 ± 0.01 m/s; 3xTgAD, control 0.29 ± 0.01 m/s, high-fat 0.29 ± 0.01 m/s; $p < 0.001$, 3xTgAD versus non-Tg on either diet). At all other ages (7–8, 11–12, and 15–16 months) there was no longer an effect of genotype on swim speed. There was no effect diet on swim speed in all groups of mice at all ages (data not shown).

3.3. High-fat diet had no effect on Aβ and tau pathology in 3xTgAD mice

To assess the effect of a high-fat diet on Aβ peptide deposition, sections from all mice were stained with 6E10, an antibody that recognizes amino acid residue 1–16 of beta-amyloid. No extracellular Aβ plaques were detected in the brains of control or high-fat fed 3xTgAD mice at the age of 3–4 and 7–8 months but by the age of 11–12 months, occasional Aβ plaques were detected in the hippocampus of 3xTgAD mice that were more prevalent at the age of 15–16 months (Table 2 and Fig. 6A). A high-fat diet had no effect on the Aβ plaque burden in the hippocampus of 3xTgAD mice at the age of 11–12 or 15–16 months (Table 2 and Fig. 6A). No extracellular Aβ plaques were detected in the brains of non-Tg mice or in the cortex and amygdala of 3xTgAD mice on either a control or high-fat diet.

No cells positive for hyperphosphoryated tau were found in the brain of 3 to 4-month-old 3xTgAD mice. At the age of 7–8, 11–12, and 15–16 months, tau-positive cells were detected in the hippocampus and amygdala. In all brain regions, there was no

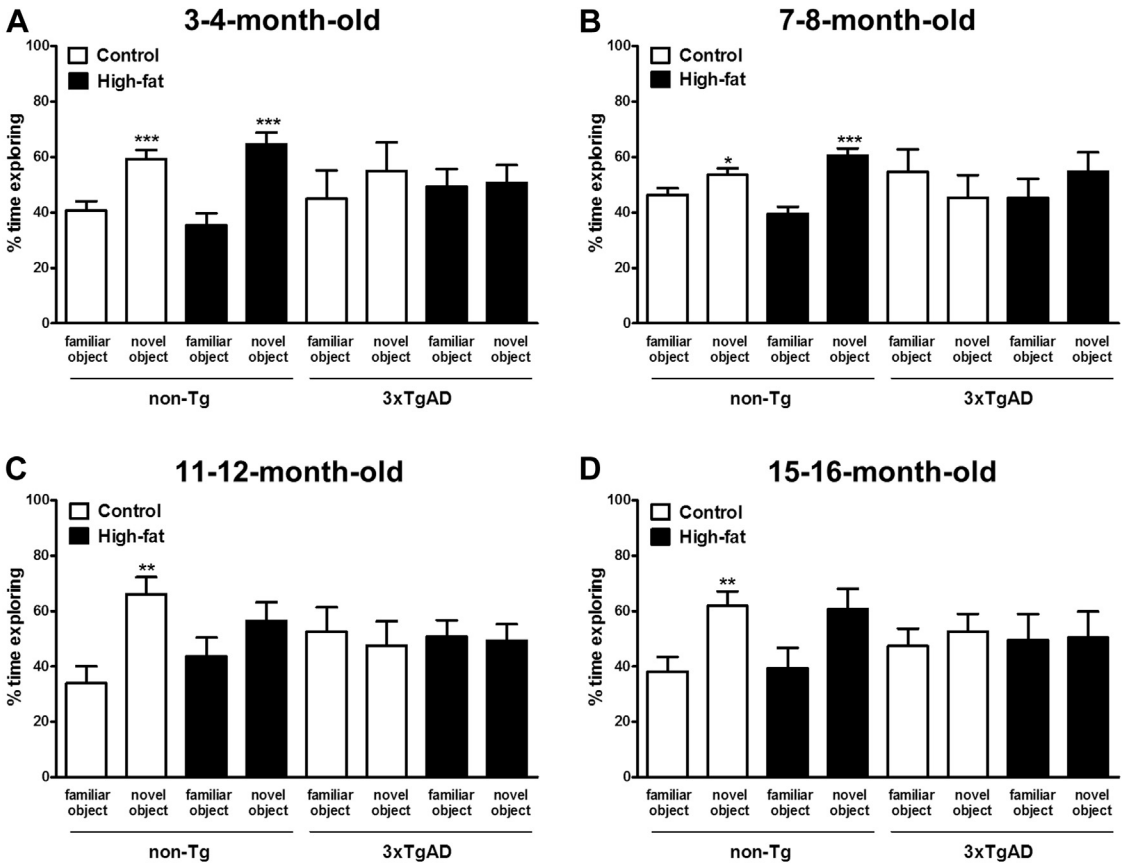

Fig. 4. High-fat diet impairs learning in the novel object recognition test in non-Tg mice. Male 3xTgAD and non-Tg control mice were maintained on either a control or high-fat diet from the age of 8 weeks. Cognitive function was assessed in the novel object recognition test in separate groups of mice at the age of 3–4 (A), 7–8 (B), 11–12 (C), and 15–16 (D) months. During phase 1, the mice were placed in an arena for 10 minutes with 2 identical novel objects (data not shown). During phase 2, after an interval of 1 hour, the mice were placed back into the arena with one familiar object (presented in phase 1) and one novel object for 4 minutes and percentage time exploring objects was compared. Data are means ± SEM. n = 6–12/group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.01$ for novel versus familiar object; Student t test. Abbreviation: SEM, standard error of the mean.

significance difference in the number of tau-positive cells between 3xTgAD mice fed a control or high-fat diet (Table 2 and Fig. 6B). No hyperphosphoryated tau was detected in the brains of non-Tg mice or in the cortex of 3xTgAD mice on either a control or high-fat diet.

The level of Aβ oligomers was analyzed in the hippocampus of 3xTgAD mice by ELISA. There was no difference in the amount of oligomers detected between control and high-fat fed 3xTgAD mice at all ages tested (Table 2).

3.4. High-fat diet increases microglia activation in non-Tg and 3xTgAD mice

At the age of 15–16 months, a significant ($p < 0.05$–0.001) increase in the number of activated microglia were detected in the hippocampus of 3xTgAD mice on either a control or high-fat diet when compared with non-Tg mice. A high-fat diet increased the number of activated microglia at the age of 3–4 months in 3xTgAD mice and at the age of 15–16 months in non-Tg mice (Fig. 7). The activated microglia were found in the same region of the hippocampus (dorsal subiculum) as to where the Aβ plaques were detected in the 3xTgAD mice (Fig. 6A).

4. Discussion

The present study is the first to assess longitudinally the effect of a high-fat diet in both control non-Tg and 3xTgAD mice, and to simultaneously measure cognitive function and pathology. We demonstrate that a high-fat diet increases the onset and severity of memory deficits in 3xTgAD mice. The effects of a high-fat diet on cognition in 3xTgAD mice are independent of an effect on AD neuropathology as no difference was observed in the deposition of Aβ and tau. Furthermore, high-fat feeding also caused memory impairments in control non-Tg mice.

Overconsumption of diets high in saturated fats is a common problem in developed countries and is likely the primary cause of obesity. Obesity is associated with an increased risk of several peripheral diseases but recent evidence suggests that obesity can also affect the brain. Midlife obesity and consumption of diets high in fat are linked to a greater risk of AD in humans (Beydoun et al., 2008; Eskelinen et al., 2008; Fitzpatrick et al., 2009; Grant, 1999; Gustafson et al., 2003; Hassing et al., 2009; Kalmijn et al., 1997; Kivipelto et al., 2005; Laitinen et al., 2006; Luchsinger et al., 2002; Profenno et al., 2009; Rosengren et al., 2005; Whitmer et al., 2005, 2007, 2008). Several studies have shown that obesity can also affect the brain in nondemented subjects and is linked with structural abnormalities, such as reduced brain and hippocampal volume, atrophy (e.g., temporal lobe), and white matter lesions (Gazdzinski et al., 2008; Gustafson et al., 2004a, 2004b; Jagust et al., 2005; Ward et al., 2005). Furthermore, central obesity has been linked with poorer cognition in elderly nondemented individuals (Jeong et al., 2005). These studies in obese humans suggest that increased adiposity can influence key areas in the brain that regulate memory and are affected in AD.

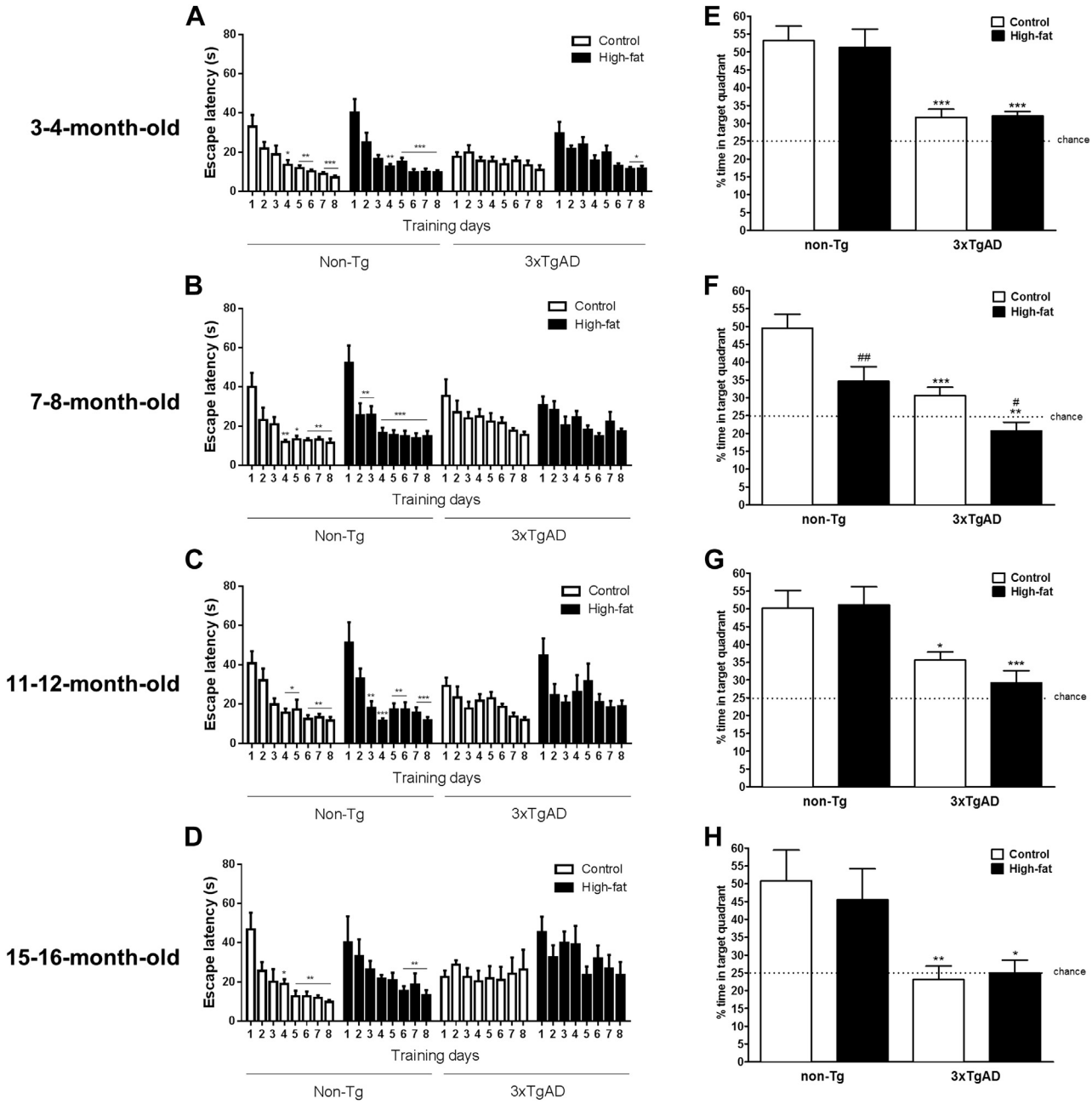

Fig. 5. High-fat diet impairs learning in the Morris water maze in 3xTgAD and non-Tg mice. Male 3xTgAD and non-Tg control mice were maintained on either a control or high-fat diet from the age of 8 weeks. Cognitive function was assessed in the Morris water maze in separate groups of mice at the age of 3–4 (A), 7–8 (B), 11–12 (C), and 15–16 (D) months. Mice were given 4 trials a day for 8 days of submerged platform training in the MWM (A–D). Twenty-four hours after the final trial the mice were given a probe test with no platform (E–H). Data are mean +/− SEM. For escape latency, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ day 1 versus days 2–8. For percentage time in target, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ versus non-Tg mice on the same diet, # $p < 0.05$, ## $p < 0.01$ versus control-fed mice of the same genotype. Abbreviations: MWM, Morris water maze; SEM, standard error of the mean.

To date most studies on the effect of a high-fat diet on memory in rodents have assessed spatial memory only and have usually evaluated memory in one behavioral test. The present study used a battery of tests to assess the effect of high-fat consumption on spatial and nonspatial memory in mice. Regardless of the test used a high-fat diet impaired both spatial and nonspatial memory in non-Tg and 3xTgAD mice. In some behavioral tests a high-fat diet had a rapid effect on memory, although in other tests an effect was apparent at a later time point. A difference in the timing of the detrimental effect of a high-fat diet on cognition has been reported previously and is dependent on the use of spatial versus nonspatial memory tests (Kanoski and Davidson, 2011). These data therefore suggest that different modes of memory are differentially sensitive to the effects of a high-fat diet. Although the present study demonstrates that a high-fat diet caused a rapid but also long-lasting impairment in cognition some of these effects, especially in the non-Tg mice, were transient although this was not observed in all behavioral tests. The transient nature of the high-fat diet induced memory impairment is currently not understood. However, overall these data demonstrate that the choice of timing and the type of behavioral test used is therefore important when assessing the cognitive effects of high-fat consumption in mice.

Table 2
A high-fat diet had no effect on Alzheimer's disease neuropathology in 3xTgAD mice

	Cortex		Hippocampus		Amygdala	
	Control	High-fat	Control	High-fat	Control	High-fat
Aβ						
Plaque burden						
3–4 mo	nd	nd	nd	nd	nd	nd
7–8 mo	nd	nd	nd	nd	nd	nd
11–12 mo	nd	nd	680 ± 183	585 ± 264	nd	nd
15–16 mo	nd	nd	2713 ± 458	6177 ± 1845	nd	nd
Oligomers (nmol/L)						
3–4 mo	—	—	2.7 ± 0.4	3.9 ± 0.4	—	—
7–8 mo	—	—	4.2 ± 0.4	3.1 ± 0.2	—	—
11–12 mo	—	—	3.6 ± 0.4	3.1 ± 0.3	—	—
15–16 mo	—	—	3.8 ± 0.5	4.1 ± 0.4	—	—
Tau						
3–4 mo	nd	nd	nd	nd	nd	nd
7–8 mo	nd	nd	1 ± 1	nd	2 ± 2	1 ± 1
11–12 mo	nd	nd	7 ± 1	8 ± 3	2 ± 1	1 ± 1
15–16 mo	nd	nd	8 ± 6	3 ± 1	9 ± 4	12 ± 5

3xTgAD mice were maintained on a control or high-fat diet for those aged 3–4, 7–8, 11–12, and 15–16 months. Immunohistochemistry for Aβ or hyperphosphorylated tau was performed using 6E10 and AT8 antibodies, respectively. The average number of cells per section positive for tau were counted in the cortex, hippocampus, and amygdala. The extracellular Aβ plaque burden in the hippocampus was assessed and data are expressed as average plaque area/section (μm^2). The expression of Aβ oligomers was analyzed by ELISA in the hippocampus. Data are mean ± SEM, n = 6–12/group.
Key: Aβ, amyloid beta; ELISA, enzyme-linked immunosorbent assay; nd, none detected; —, not analyzed; SEM, standard error of the mean.

In the present study the detrimental effect of a high-fat diet on memory in 3xTgAD mice was not associated with a change in the extent of Aβ and tau deposition. In support, not all studies reporting a worsening of memory in AD mice fed a high-fat diet have observed an effect on pathology (Herculano et al., 2013; Phivilay et al., 2009). However, several studies have shown that high-fat feeding can increase soluble Aβ and/or Aβ plaques in the brain of AD mice (Ho et al., 2004; Julien et al., 2010; Maesako et al., 2012a; Pedrini et al., 2009). Aβ oligomers are currently thought to be the key species involved in Aβ toxicity (Lesne et al., 2006; Shankar et al., 2008; Walsh et al., 2002) but in the present study no change in expression of soluble oligomers was detected in the hippocampus of 3xTgAD mice on a high-fat diet at any time point. High-fat consumption also caused memory deficits in control non-Tg mice, which do not present with Aβ or tau neuropathology. Furthermore, intermittent fasting has been shown to reduce cognitive deficits in 3xTgAD mice without effecting Aβ and tau levels (Halagappa et al., 2007). Thus, the effects of a high-fat diet on cognition in the present study are therefore likely not because of changes in AD-related neuropathology.

High-fat feeding caused an increase in body weight to the same extent in non-Tg and 3xTgAD mice. However, after the age of 7–8 months 3xTgAD mice on a high-fat diet had higher epididymal adipose mass compared with non-Tg control mice suggesting that the metabolic response to excess calories is different in the 3xTgAD mice. The present data also confirms a previous study showing that early in life 3xTgAD mice on a control diet weigh more and have higher food consumption than non-Tg controls (Knight et al., 2012), although food intake was not assessed here. This increased body weight shown here was not because of greater adipose tissue deposition, at least in the epididymal depots although other visceral depots and subcutaneous adipose remains to be assessed. It is likely therefore that other factors contribute to the greater body weight as 3xTgAD mice have longer body lengths and higher spleen mass (unpublished data). Altered metabolism has also been demonstrated in the 3xTgAD mouse as after the age of 12 months, these mice are no longer heavier than the control non-Tg mice even though food intake is still increased, an observation that might be because of a higher metabolic rate (Adebakin et al., 2012; Knight et al., 2012). However, it is likely that body weight and/or obesity per se may not be the main cause for cognitive deficits as memory deficits in high-fat fed AD mice can be reduced (with an antioxidant) without an effect on body weight (Herculano et al., 2013). Furthermore, when body weight is normalized after the high-fat diet is replaced with control diet, AD mice still experience cognitive deficits (Maesako et al., 2012b). Exercise is also more effective than diet control at reducing cognitive impairment in high-fat fed AD mice, even though exercise only induced a minor reduction in body weight (Maesako et al., 2012b). These data therefore suggest that long lasting metabolic consequences of high-fat feeding rather than body weight and/or adiposity are responsible for a reduction in cognition in mice.

The mechanisms responsible for the effect of a high-fat diet on memory remain unknown, although there are several possibilities. Neuroinflammation and oxidative stress are pathologic features of AD that are proposed to play a key role in the disease pathogenesis (Johnston et al., 2011; Verri et al., 2012; Wyss-Coray and Rogers, 2012). Microglia are the brain-resident "immune" cells and are proposed to play a key role in many neurodegenerative conditions and particularly in AD (Wyss-Coray and Rogers, 2012). Microglial activation was increased in response to a high-fat diet seen here in both 3xTgAD and non-Tg control mice, and also in other mouse models of AD (Herculano et al., 2013). Vascular inflammation might also be important in the effects of obesity on cognition as diets high in fat also increase expression of vascular inflammatory markers in AD mice (Herculano et al., 2013). Furthermore, when AD transgenic mice (APP23) are

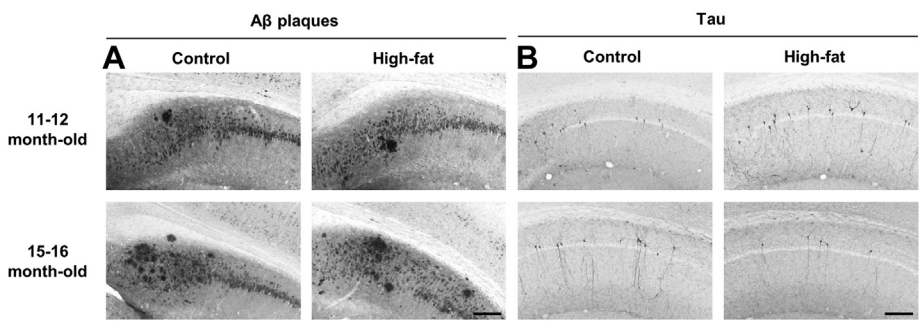

Fig. 6. High-fat diet has no effect on Aβ or tau neuropathology in the hippocampus of 3xTgAD mice. Male 3xTgAD and non-Tg control mice were maintained on either a control or high-fat diet from the age of 8 weeks until the age of 11–12 and 15–16 months. Immunohistochemistry for Aβ (A) or hyperphosphorylated tau (B) was performed using 6E10 and AT8 antibodies, respectively. Representative sections for 3xTgAD mice are shown for the hippocampus indicating the extracellular Aβ plaque burden and tau-positive neurons. Quantification is presented in Table 2. Scale bars 200 μm. Abbreviation: Aβ, beta amyloid.

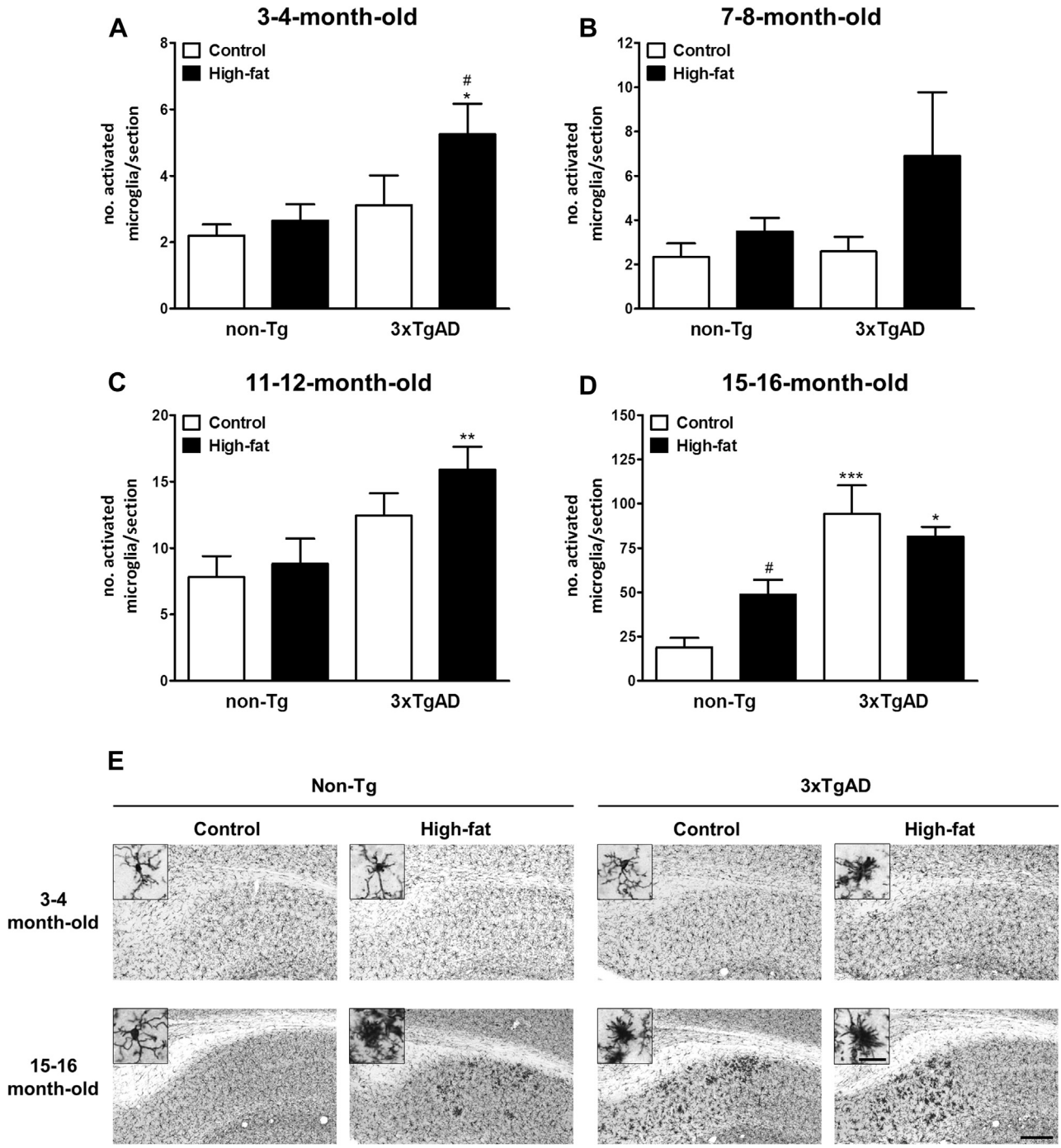

Fig. 7. High-fat diet increases microglia activation in the hippocampus of 3xTgAD and non-Tg mice. Male 3xTgAD and non-Tg control mice were maintained on either a control or high-fat diet from the age of 8 weeks until the age of 3–4 (A), 7–8 (B), 11–12 (C), and 15–16 (D) months. Microglia activation were identified by increased Iba1 immunopositivity, enlarged and/or irregular cell bodies, thickened, partial or complete loss of processes and were seen after high-fat feeding in 3xTgAD mice at the age of 3–4 months and non-Tg mice at the age of 15–16 months. An increase in microglia activation was also observed in control fed 3xTgAD mice over time. (E) Representative photomicrographs in the hippocampus from 3–4 and 15 to 16-month-old mice. Scale bars 200 μm and 25 μm (insets). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ versus non-Tg group on the same diet; # $p < 0.05$ versus control fed of the same genotype.

crossed with an obese mouse model (*ob/ob*) the resulting offspring (APP^{+}-*ob/ob*) show worse cognitive deficits and vascular inflammation that appear before significant Aβ deposition (Takeda et al., 2010). Oxidative stress is also a key feature of AD and can be increased in response to dysregulated inflammation. Heighted oxidative stress is observed after short-term high-fat feeding in both AD and control mice and antioxidants have been shown to be reduce the memory deficits in AD mice fed a high-fat diet (Herculano et al., 2013).

The data in the present study indicate that high-fat diets impair memory in control and AD mice. It remains to be determined if the same or different mechanisms impact on memory in control and 3xTgAD mice, though the underlying mechanisms do not involve exacerbation of Aβ and tau pathology.

Disclosure statement

The authors declare no conflicts of interest.

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References

Adebakin, A., Bradley, J., Gumusgoz, S., Waters, E.J., Lawrence, C.B., 2012. Impaired satiation and increased feeding behaviour in the triple-transgenic Alzheimer's disease mouse model. PLoS. One 7, e45179.

Beydoun, M.A., Beydoun, H.A., Wang, Y., 2008. Obesity and central obesity as risk factors for incident dementia and its subtypes: a systematic review and meta-analysis. Obes. Rev. 9, 204–218.

Billings, L.M., Oddo, S., Green, K.N., McGaugh, J.L., Laferla, F.M., 2005. Intraneuronal abeta causes the onset of early Alzheimer's disease-related cognitive deficits in transgenic mice. Neuron 45, 675–688.

Buchman, A.S., Wilson, R.S., Bienias, J.L., Shah, R.C., Evans, D.A., Bennett, D.A., 2005. Change in body mass index and risk of incident Alzheimer disease. Neurology 65, 892–897.

Drake, C., Boutin, H., Jones, M.S., Denes, A., McColl, B.W., Selvarajah, J.R., Hulme, S., Georgiou, R.F., Hinz, R., Gerhard, A., Vail, A., Prenant, C., Julyan, P., Maroy, R., Brown, G., Smigova, A., Herholz, K., Kassiou, M., Crossman, D., Francis, S., Proctor, S.D., Russell, J.C., Hopkins, S.J., Tyrrell, P.J., Rothwell, N.J., Allan, S.M., 2011. Brain inflammation is induced by co-morbidities and risk factors for stroke. Brain Behav. Immun. 25, 1113–1122.

Eskelinen, M.H., Ngandu, T., Helkala, E.L., Tuomilehto, J., Nissinen, A., Soininen, H., Kivipelto, M., 2008. Fat intake at midlife and cognitive impairment later in life: a population-based CAIDE study. Int. J. Geriatr. Psychiatry 23, 741–747.

Fitzpatrick, A.L., Kuller, L.H., Lopez, O.L., Diehr, P., O'Meara, E.S., Longstreth Jr., W.T., Luchsinger, J.A., 2009. Midlife and late-life obesity and the risk of dementia: cardiovascular health study. Arch. Neurol. 66, 336–342.

Gazdzinski, S., Kornak, J., Weiner, M.W., Meyerhoff, D.J., 2008. Body mass index and magnetic resonance markers of brain integrity in adults. Ann. Neurol. 63, 652–657.

Grant, W.B., 1997. Dietary links to Alzheimer's disease. Alzheimer's Dis. Rev. 2, 42–45.

Grant, W.B., 1999. Dietary links to Alzheimer's disease: 1999 update. J. Alzheimers Dis. 1, 197–201.

Gustafson, D., Lissner, L., Bengtsson, C., Bjorkelund, C., Skoog, I., 2004a. A 24-year follow-up of body mass index and cerebral atrophy. Neurology 63, 1876–1881.

Gustafson, D., Rothenberg, E., Blennow, K., Steen, B., Skoog, I., 2003. An 18-year follow-up of overweight and risk of Alzheimer disease. Arch. Intern. Med. 163, 1524–1528.

Gustafson, D.R., Steen, B., Skoog, I., 2004b. Body mass index and white matter lesions in elderly women. An 18-year longitudinal study. Int. Psychogeriatr. 16, 327–336.

Halagappa, V.K., Guo, Z., Pearson, M., Matsuoka, Y., Cutler, R.G., Laferla, F.M., Mattson, M.P., 2007. Intermittent fasting and caloric restriction ameliorate age-related behavioral deficits in the triple-transgenic mouse model of Alzheimer's disease. Neurobiol. Dis. 26, 212–220.

Hassing, L.B., Dahl, A.K., Thorvaldsson, V., Berg, S., Gatz, M., Pedersen, N.L., Johansson, B., 2009. Overweight in midlife and risk of dementia: a 40-year follow-up study. Int. J. Obes. (Lond) 33, 893–898.

Herculano, B., Tamura, M., Ohba, A., Shimatani, M., Kutsuna, N., Hisatsune, T., 2013. β-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. 33, 983–997.

Hiramatsu, M., Sasaki, M., Nabeshima, T., Kameyama, T., 1997. Effects of dynorphin A (1-13) on carbon monoxide-induced delayed amnesia in mice. Pharmacol. Biochem. Behav. 56, 73–79.

Ho, L., Qin, W., Pompl, P.N., Xiang, Z., Wang, J., Zhao, Z., Peng, Y., Cambareri, G., Rocher, A., Mobbs, C.V., Hof, P.R., Pasinetti, G.M., 2004. Diet-induced insulin resistance promotes amyloidosis in a transgenic mouse model of Alzheimer's disease. FASEB J. 18, 902–904.

Jagust, W., Harvey, D., Mungas, D., Haan, M., 2005. Central obesity and the aging brain. Arch. Neurol. 62, 1545–1548.

Jeong, S.K., Nam, H.S., Son, M.H., Son, E.J., Cho, K.H., 2005. Interactive effect of obesity indexes on cognition. Dement. Geriatr. Cogn. Disord. 19, 91–96.

Johnston, H., Boutin, H., Allan, S.M., 2011. Assessing the contribution of inflammation in models of Alzheimer's disease. Biochem. Soc. Trans. 39, 886–890.

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

Kalmijn, S., Launer, L.J., Ott, A., Witteman, J.C., Hofman, A., Breteler, M.M., 1997. Dietary fat intake and the risk of incident dementia in the Rotterdam Study. Ann. Neurol. 42, 776–782.

Kanoski, S.E., Davidson, T.L., 2011. Western diet consumption and cognitive impairment: links to hippocampal dysfunction and obesity. Physiol. Behav. 103, 59–68.

Kivipelto, M., Ngandu, T., Fratiglioni, L., Viitanen, M., Kareholt, I., Winblad, B., Helkala, E.L., Tuomilehto, J., Soininen, H., Nissinen, A., 2005. Obesity and vascular risk factors at midlife and the risk of dementia and Alzheimer disease. Arch. Neurol. 62, 1556–1560.

Knight, E.M., Brown, T.M., Gumusgoz, S., Smith, J.C., Waters, E.J., Allan, S.M., Lawrence, C.B., 2013. Age-related changes in core body temperature and activity in triple-transgenic Alzheimer's disease (3xTgAD) mice. Dis. Model Mech. 6, 160–170.

Knight, E.M., Verkhratsky, A., Luckman, S.M., Allan, S.M., Lawrence, C.B., 2012. Hypermetabolism in a triple-transgenic mouse model of Alzheimer's disease. Neurobiol. Aging 33, 187–193.

Laitinen, M.H., Ngandu, T., Rovio, S., Helkala, E.L., Uusitalo, U., Viitanen, M., Nissinen, A., Tuomilehto, J., Soininen, H., Kivipelto, M., 2006. Fat intake at midlife and risk of dementia and Alzheimer's disease: a population-based study. Dement. Geriatr. Cogn. Disord. 22, 99–107.

Lesne, S., Koh, M.T., Kotilinek, L., Kayed, R., Glabe, C.G., Yang, A., Gallagher, M., Ashe, K.H., 2006. A specific amyloid-beta protein assembly in the brain impairs memory. Nature 440, 352–357.

Luchsinger, J.A., Tang, M.X., Shea, S., Mayeux, R., 2002. Caloric intake and the risk of Alzheimer disease. Arch. Neurol. 59, 1258–1263.

Maesako, M., Uemura, K., Kubota, M., Kuzuya, A., Sasaki, K., Asada, M., Watanabe, K., Hayashida, N., Ihara, M., Ito, H., Shimohama, S., Kihara, T., Kinoshita, A., 2012a. Environmental enrichment ameliorated high-fat diet-induced Abeta deposition and memory deficit in APP transgenic mice. Neurobiol. Aging 33, 1011–1023.

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

McNeilly, A.D., Williamson, R., Sutherland, C., Balfour, D.J., Stewart, C.A., 2011. High fat feeding promotes simultaneous decline in insulin sensitivity and cognitive performance in a delayed matching and non-matching to position task. Behav. Brain Res. 217, 134–141.

Oddo, S., Caccamo, A., Kitazawa, M., Tseng, B.P., Laferla, F.M., 2003a. Amyloid deposition precedes tangle formation in a triple transgenic model of Alzheimer's disease. Neurobiol. Aging 24, 1063–1070.

Oddo, S., Caccamo, A., Shepherd, J.D., Murphy, M.P., Golde, T.E., Kayed, R., Metherate, R., Mattson, M.P., Akbari, Y., Laferla, F.M., 2003b. Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular Abeta and synaptic dysfunction. Neuron 39, 409–421.

Panza, F., Solfrizzi, V., Colacicco, A.M., D'Introno, A., Capurso, C., Torres, F., Del Parigi, A., Capurso, S., Capurso, A., 2004. Mediterranean diet and cognitive decline. Public Health Nutr. 7, 959–963.

Paxinos, G., Franklin, K., 2001. The Mouse Brain in Stereotaxic Coordintes. Academic Press, London, UK.

Pedrini, S., Thomas, C., Brautigam, H., Schmeidler, J., Ho, L., Fraser, P., Westaway, D., Hyslop, P.S., Martins, R.N., Buxbaum, J.D., Pasinetti, G.M., Dickstein, D.L., Hof, P.R., Ehrlich, M.E., Gandy, S., 2009. Dietary composition modulates brain mass and solubilizable Abeta levels in a mouse model of aggressive Alzheimer's amyloid pathology. Mol. Neurodegener. 4, 40.

Phivilay, A., Julien, C., Tremblay, C., Berthiaume, L., Julien, P., Giguere, Y., Calon, F., 2009. High dietary consumption of trans fatty acids decreases brain docosahexaenoic acid but does not alter amyloid-beta and tau pathologies in the 3xTg-AD model of Alzheimer's disease. Neuroscience 159, 296–307.

Pistell, P.J., Morrison, C.D., Gupta, S., Knight, A.G., Keller, J.N., Ingram, D.K., Bruce-Keller, A.J., 2010. Cognitive impairment following high fat diet consumption is associated with brain inflammation. J. Neuroimmunol. 219, 25–32.

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

Rosengren, A., Skoog, I., Gustafson, D., Wilhelmsen, L., 2005. Body mass index, other cardiovascular risk factors, and hospitalization for dementia. Arch. Intern. Med. 165, 321–326.

Serrano-Pozo, A., Frosch, M.P., Masliah, E., Hyman, B.T., 2011. Neuropathological alterations in Alzheimer disease. Cold Spring Harb. Perspect. Med. 1, a006189.

Shankar, G.M., Li, S., Mehta, T.H., Garcia-Munoz, A., Shepardson, N.E., Smith, I., Brett, F.M., Farrell, M.A., Rowan, M.J., Lemere, C.A., Regan, C.M., Walsh, D.M., Sabatini, B.L., Selkoe, D.J., 2008. Amyloid-beta protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. Nat. Med. 14, 837–842.

Stewart, R., Masaki, K., Xue, Q.L., Peila, R., Petrovitch, H., White, L.R., Launer, L.J., 2005. A 32-year prospective study of change in body weight and incident dementia: the Honolulu-Asia Aging Study. Arch. Neurol. 62, 55–60.

Takeda, S., Sato, N., Uchio-Yamada, K., Sawada, K., Kunieda, T., Takeuchi, D., Kurinami, H., Shinohara, M., Rakugi, H., Morishita, R., 2010. Diabetes-accelerated memory dysfunction via cerebrovascular inflammation and Abeta deposition in an Alzheimer mouse model with diabetes. Proc. Natl. Acad. Sci. U.S.A 107, 7036–7041.

Verri, M., Pastoris, O., Dossena, M., Aquilani, R., Guerriero, F., Cuzzoni, G., Venturini, L., Ricevuti, G., Bongiorno, A.I., 2012. Mitochondrial alterations,

oxidative stress and neuroinflammation in Alzheimer's disease. Int. J. Immunopathol. Pharmacol. 25, 345–353.
Vorhees, C.V., Williams, M.T., 2006. Morris water maze: procedures for assessing spatial and related forms of learning and memory. Nat. Protoc. 1, 848–858.
Wall, P.M., Messier, C., 2002. Infralimbic kappa opioid and muscarinic M1 receptor interactions in the concurrent modulation of anxiety and memory. Psychopharmacology (Berl) 160, 233–244.
Walsh, D.M., Klyubin, I., Fadeeva, J.V., Cullen, W.K., Anwyl, R., Wolfe, M.S., Rowan, M.J., Selkoe, D.J., 2002. Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. Nature 416, 535–539.
Ward, M.A., Carlsson, C.M., Trivedi, M.A., Sager, M.A., Johnson, S.C., 2005. The effect of body mass index on global brain volume in middle-aged adults: a cross sectional study. BMC Neurol. 5, 23.
Whitmer, R.A., Gunderson, E.P., Barrett-Connor, E., Quesenberry Jr., C.P., Yaffe, K., 2005. Obesity in middle age and future risk of dementia: a 27 year longitudinal population based study. BMJ 330, 1360.
Whitmer, R.A., Gunderson, E.P., Quesenberry Jr., C.P., Zhou, J., Yaffe, K., 2007. Body mass index in midlife and risk of Alzheimer disease and vascular dementia. Curr. Alzheimer Res. 4, 103–109.
Whitmer, R.A., Gustafson, D.R., Barrett-Connor, E., Haan, M.N., Gunderson, E.P., Yaffe, K., 2008. Central obesity and increased risk of dementia more than three decades later. Neurology 71, 1057–1064.
Winocur, G., Greenwood, C.E., 2005. Studies of the effects of high fat diets on cognitive function in a rat model. Neurobiol. Aging 26 (Suppl. 1), 46–49.
Wyss-Coray, T., Rogers, J., 2012. Inflammation in Alzheimer disease-a brief review of the basic science and clinical literature. Cold Spring Harb. Perspect. Med. 2, a006346.

Chapter 3

Earlier memory impairment after a high-fat diet in male versus female mice is associated with hyperinsulinemia

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Earlier memory impairment after a high-fat diet in male versus female mice is associated with hyperinsulinemia

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Keywords: obesity; Alzheimer’s disease; control non-transgenic, Non-Tg; triple-transgenic AD, 3xTgAD; high-fat diet; memory; insulin, hyperinsulinemia.

Abstract

Midlife obesity is considered a risk factor for the development of Alzheimer's disease (AD) later in life and experimental obesity, due to diets rich in fatty acids and cholesterol, leads to cognitive deficits in rodents. However, experimental studies to date assess effects of high-fat diets on memory mostly in male rodents, with effects in females being less clear. Moreover, it is known that sex hormones, as testosterone and estrogen, are important in the progression of AD and can have an effect on memory. Therefore, the aim of this study was to test the effect of a high-fat diet on memory in both male and female control non-transgenic (Non-Tg) and triple-transgenic AD (3xTgAD) mice using a battery of behavioural tests. Non-Tg and 3xTgAD mice were placed on either a control (12% fat) or high-fat diet (60% fat) for a period of 7 weeks (short-term) or 12 months (long-term). Male Non-Tg mice were more susceptible to diet-induced obesity after 7 weeks of high-fat diet, presenting greater gains in body weight, adiposity and increased insulin levels, which were associated with memory deficits in the novel object recognition test. After 12 months of high-fat diet, both male and female Non-Tg mice presented memory deficits in the novel object recognition and Y-maze tests, both groups showing increased insulin levels. A high-fat diet had no further effect on existing memory deficits in male or female 3xTgAD mice at 6 or 12 months, and in 3xTgAD mice a high-fat diet had no effect on insulin levels at any time point when compared with control fed mice. Overall these results demonstrate that Non-Tg male mice exhibit earlier deficits in memory in response to a high-fat diet, an effect that was associated with an increase in insulin. However, in 3xTgAD mice a high-fat diet had no effect on memory or insulin levels. These data therefore suggest that hyperinsulinemia might be important in the detrimental effect of a high-fat diet on memory.

Introduction

Consumption of diets high in fat, such as western diets, are increasing worldwide and contributing to an overweight and obese population. Obesity, which is defined by an increase in adipose tissue, has been associated with effects on cognitive function including impairment in memory [1-6]. Being obese is also considered a risk factor for developing dementia later in life, and has been reported to increase the risk of Alzheimer's disease (AD) [7-14]. Obesity is a component of the metabolic syndrome, characterised as a cluster of metabolic abnormalities, which includes visceral adiposity, hypertension and hyperinsulinemia, amongst other features [15]. Like obesity, metabolic syndrome has also been associated with cognitive impairment and AD [16-18]. Obese young adults have changes in brain morphology, such as loss of neurones and myelin, in regions that mediate cognitive function similar to those affected in AD patients [19, 20]. Experimental studies also show that high-fat diets can impair learning and memory in rodents [6, 21-30] and that body weight gain is associated with selective hippocampal damage [6, 31, 32]. Diets high in fat can also worsen memory deficits in mouse models of AD [33-38]. These data suggest a strong association between obesity and cognitive dysfunction.

The majority of experimental studies showing negative effects of high-fat diets/obesity on cognition are in male rodents and limited to just one time point, typically 6 months on diet or less. It is known that the metabolic response to a high-fat diet can differ between males and females and in general, women have more body fat and are more susceptible to weight gain than men [39]. Moreover, sex hormones, in particular ovarian hormones such as estrogen, are known to have an important role not only in AD but also in memory mechanisms. Estrogen has a neurotrophic action in brain regions that are involved in memory and learning and in women with AD estrogen levels are decreased, which suggests that estrogen can be neuroprotective [40].

Several recent experimental studies show gender differences in the metabolic response to diet-induced obesity in mice [41-48] and variation in the extent of obesity and/or obesity-related changes, such as insulin resistance, between sexes might impact on how memory is affected by high-fat diets. Indeed, gender differences in the metabolic response after high-fat feeding is reported to produce a greater deficit in learning and synaptic plasticity in male compared to female

mice [43]. However, no study to date has been performed to assess the impact of gender on the effect of a high-fat diet on memory in cognitively normal versus cognitively impaired rodents including models of AD.

Therefore, the aim of this study was to evaluate the effect of a high-fat diet on memory in male and female control non-transgenic (Non-Tg) mice and in the triple transgenic mouse model of AD (3xTgAD). We have shown previously that, depending on the behavioural test used, the negative effects of a high-fat diet on memory in male Non-Tg and 3xTgAD mice can be transient and/or the age at which cognitive impairment is detected can vary [33]. Thus, groups of male and female Non-Tg and 3xTgAD mice were placed on either a control or high-fat diet and the metabolic response and memory (using several behavioural tests) were assessed in separate groups of animals after short- (7 weeks) and long-term (12 months) diet.

Methods

Animals and diet

An in-house colony of male and female 3xTgAD mice, expressing mutant $PS1_{M146V}$, APP_{SWE} and Tau_{P301L}, and Non-Tg controls, on a C57BL6 x 129SV background, was established from breeding pairs originally supplied by Frank LaFerla (Irvine, CA, USA) [49]. Mice were kept in standard housing conditions (humidity 50%-60%, temperature 21 ± 1˚C, 12:12 hour light-dark) and given *ad libitum* access to food and water. After weaning, male and female littermates for both Non-Tg and 3xTgAD mice were housed separately and kept on standard rodent chow (BK001, Special Diets Services, UK) until 8 weeks of age. Groups of mice were then randomly allocated to either control (12% energy from fat, 5% fat content by weight, 0.78% saturated fatty acids, 58G7, Test Diets) or high-fat diet (60% energy from fat, 35% fat content by weight, 13% saturated fatty acids, 58G9, Test Diets) and maintained on their respective diets for 7 weeks (n = 9-12/group) or 12 months (n = 9-12/group). Body weight was assessed at baseline, before placement on diet, and monitored at 7 weeks and 12 months of diet in the respective groups of mice. Blood pressure and behaviour were assessed at 7 weeks and in a separate group of mice at 6 and 12 months (Figure 1). All animal

experiments were carried out in accordance with the United Kingdom Animals (Scientific Procedures) Act 1986 and approved by the Home Office and the local Animal Ethical Review Group, University of Manchester.

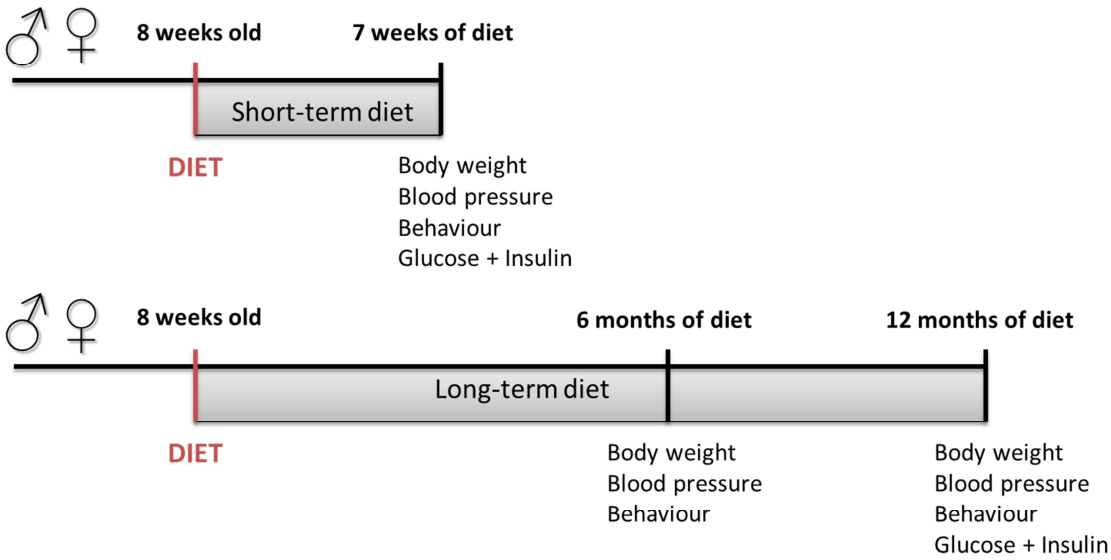


Figure 1. Experimental design. Non-Tg and 3xTgAD male and female mice were kept on a standard rodent chow diet until 8 weeks of age. Separate groups of animals were then randomly allocated to either control or high-fat diet for 7 weeks (short-term diet) or 12 months (long-term diet). Body-weight, blood pressure and behaviour were assessed at the end of each feeding period and at 6 months for the long-term diet group.

Blood pressure

Systolic blood pressure and heart rate were assessed using a non-invasive blood pressure analyser with a specific mouse tail cuff adapter (Model BP-2000-M-2, Visitech Systems, Inc, USA). One day prior to measurements, mice were habituated to the procedure for 5 minutes to reduce stress and anxiety. On the day of the assessment the animals were placed in the room and allowed to acclimatise for 10 minutes. A total of 20 readings of systolic blood pressure (mmHg) and heart rate (beats/minute, bpm) were taken and the average of the last 10 measurements calculated.

Behaviour

Mice were subjected to open-field, smell recognition, novel object recognition (NOR) and Y-maze spontaneous alternation tests. To decrease anxiety, all animals were allowed to habituate to the test room for 30 minutes prior to behavioural tests. All experiments were performed between 9am and 5pm in the same testing room. Each test was performed on a different day and the animal order was randomised between groups. To avoid distress and anxiety, behavioural tests were performed on separate days for male and female mice. All apparatus were cleaned between animals and tests with 70% ethanol. The subsequent analysis of all behaviour data was performed blinded to genotype and diet.

Open-field test

General motor function and exploratory activity was assessed in the open-field by placing the mouse in an unfamiliar environment, which consisted of a square opaque Perspex box (45 cm x 45 cm x 30cm). The animal was positioned in the centre of the arena and allowed to explore for 5 minutes. Behaviour was recorded with a video camera and Any-Maze Tracking software (Stoelting Co, Dublin, Ireland) was used to track movement. Total time moving was measured and % time moving was calculated.

Smell and novel object recognition tests

To evaluate recognition memory, based on the spontaneous tendency of the animal to spend more time examining a smell/novel object compared to a familiar one [50], smell recognition and NOR tests were used as described previously [33]. Briefly, for both tests, all animals were allowed to habituate to a black opaque polycarbonate circular arena (diameter, 30 cm x height, 21 cm) for 5 minutes over 2 days. On the third day, mice were placed in the centre of the arena and allowed to explore 2 identical scented balls, for the smell recognition test, or 2 identical objects for the novel object recognition test, for 10 minutes (phase 1). The scented balls (Chad Valley, UK) were filled with cotton wool and 0.5 ml of scent (orange, lemon, vanilla, almond, peppermint or eucalyptus, Dr Oetker Ltd, UK) was distributed into the balls via small holes. Mice were then removed and one of

the scented balls was replaced by a ball containing a novel scent or for the NOR test one of the objects was replaced with a novel object that varied in shape and colour. After a delay of 3 minutes for smell recognition or 1 hour for NOR the mice were placed back into the arena and allowed to explore for 4 minutes (phase 2). All experiments were recorded with a camera (Sanyo Xacti VPC-C4, SANYO Fisher, CA, USA) and MP4 video-clips were converted to an AVI format using Pazera MP4 to AVI converter 1.3 (Pazera-Software, Poland). The time (seconds) spent exploring the objects was measured using SR Ethovision XT 8.5 (Noldus, Wageningen, The Netherlands) whilst time spent exploring the scented balls was calculated manually by observing each individual video. Exploration was defined as the amount of time that the animals spent with their nose within 2 cm in the direction of the smell/object. The percentage of time spent exploring was calculated for each phase of the test.

Y-maze spontaneous alternation test

Short-term working memory was assessed using the Y-maze spontaneous alternation test. A black opaque Perspex Y-shaped maze with three arms (A, B and C) was used and a visual cue was located at each end of the arm (arm dimensions; 15 cm x 10 cm x 10 cm). Each animal was placed into the maze facing the end of arm A and allowed to explore for 8 minutes. The number of arm entries (when the entire body of the animal was inside the arm space) made by each animal was recorded. Spontaneous alternation was defined as three consecutive entries in different arms (triplets) in a random order and the percentage number of alternations between arms was calculated as defined previously [33].

Tissue collection

At the end of the feeding period, mice were terminally anesthetized with 3.5% isoflurane (30% O_2 and 70% N_2O), blood was taken from the heart using 3.8% sodium citrate as an anticoagulant and plasma obtained after centrifugation (1200 *g*, 10 min) was stored at -80°C until assay. To remove blood, animals were then perfused transcardially with 0.9% saline. Total gonadal white adipose tissue, liver and spleen were dissected and weighed.

Glucose and insulin measurements

Non-fasting blood glucose was measured in samples of cardiac blood by an Accu-Chek Diabetes kit (Roche Diagnostics, Indianapolis, IN, USA). Insulin levels were measured in plasma samples using an ultra-sensitive insulin mouse ELISA kit (Crystal Chem Inc, IL, USA) according to the manufacture's protocol.

Statistical analyses

All data are expressed as the mean ± standard error of the mean (SEM). Body and organ weights, glucose, insulin, systolic blood pressure, heart rate, and data from the open-field and Y-maze spontaneous alternation tests were analysed using a two-way analysis of variance (ANOVA) followed by Bonferroni´s post-hoc test using SigmaPlot (Systat Software, Inc., San Jose, CA, USA). For the smell recognition and novel object tests a Student's *t* test was used (GraphPad Prism 6, GraphPad Software, Inc., CA, USA). Statistical significance was taken when $p < 0.05$.

Results

High-fat diet leads to a greater increase in gonadal fat content in male Non-Tg mice after 7 weeks on diet

After 7 weeks, a high-fat diet led to a greater increase in body weight in male Non-Tg and male 3xTgAD mice. In male Non-Tg mice, change in body weight was approximately 148% while in male 3xTgAD mice a 44% change was observed (Figure 2A). Male 3xTgAD on a control diet gained more weight than Non-Tg matched controls (Figure 2A), a phenotype that was not observed in females (Figure 2B). In female Non-Tg mice only a 46% increase in body weight was observed with 7 weeks of high-fat diet while in female 3xTgAD mice a 116% was seen (Figure 2B). Increases in body weight in male Non-Tg mice after 7 weeks on high-fat diet were accompanied by a 203% increase in gonadal fat content compared to just a 110% increase in females (Table 1). In 3xTgAD mice, gonadal fat content increased 81% in females and 110% in males after a high-fat diet (Table 1). At

this time no changes in liver weight were observed between genotypes or diets (Table 1). Both male and female 3xTgAD mice fed on a control or high-fat diet had bigger spleens than the respective Non-Tg control mice (Table 1).

Body weight continued to increase with a high-fat diet after 6 months in both male and female Non-Tg and 3xTgAD mice. In male Non-Tg mice in response to a high-fat diet there was a 69% increase in change of body weight from baseline (Figure 2C), while in male 3xTgAD mice 90% increase was observed (Figure 2C). Female Non-Tg mice had an increase of 135% on body weight (Figure 2D) and female 3xTgAD had 163% due to high-fat feeding (Figure 2D).

After 12 months, male and female Non-Tg and 3xTgAD mice fed on a high-fat diet were significantly heavier than control fed animals. In males, change in body weight increased by 97% in Non-Tg and 120% in the 3xTgAD mice after high-fat feeding (Figure 2E). In female mice a 128% change in body weight was observed in the Non-Tg and 123% in the 3xTgAD with a high-fat diet (Figure 2F). Gonadal fat weight was also increased after 12 months on a high-fat diet in both male and female 3xTgAD mice (274% and 335% respectively) but only female Non-Tg mice on the high-fat diet showed an increase in adipose tissue (217%) (Table 1). A high-fat diet also led to heavier livers in both male and female Non-Tg mice (111% and 21.6% respectively) but only in male 3xTgAD mice (24.5% increase) (Table 1). Livers from high-fat diet fed male and female Non-Tg and male 3xTgAD mice were paler and contained fat deposits. Male 3xTgAD mice on both diets had an increase in spleen weight compared to Non-Tg controls. In female 3xTgAD mice, a high-fat diet led to a 75% increase in spleen weight compared to 3xTgAD fed on a control diet (Table 1).

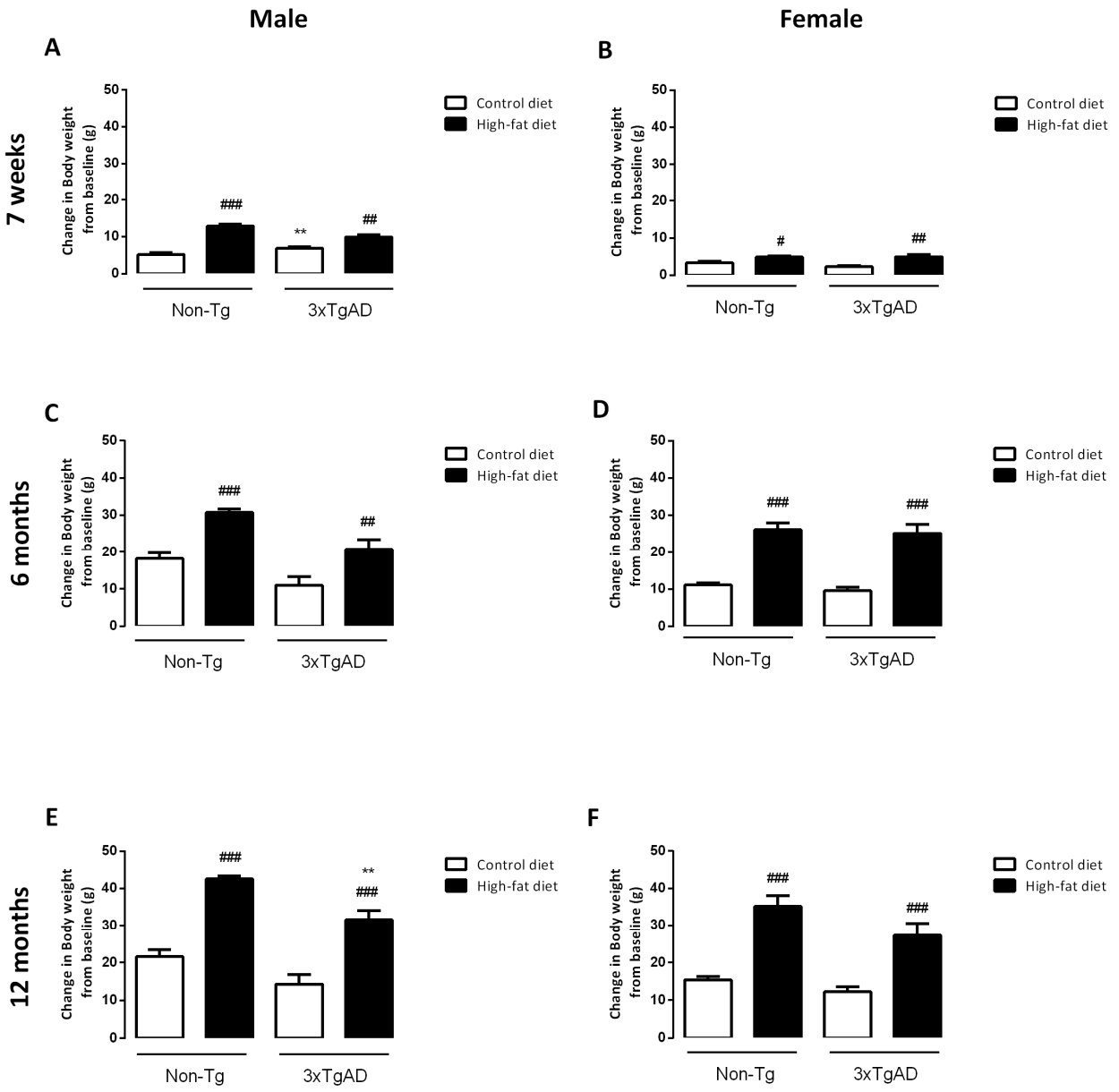


Figure 2. High-fat diet increases body weight in male and female Non-Tg and 3xTgAD mice. Groups of male and female Non-Tg control and 3xTgAD mice were maintained on a control or high-fat diet from 8 weeks of age. Body weight was assessed at baseline (before diet) and after 7 weeks and, in a separate group of animals, after 6 and 12 months on diet. Change in body weight from baseline was calculated (g). Data are mean ± SEM, n = 9-12/group. # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ versus control diet of the same genotype. ** $p < 0.01$ versus Non-Tg on the same diet. Two-way ANOVA with Bonferroni's post-hoc analysis.

Table 1

Organs weight of male and female Non-Tg control and 3xTgAD mice in response to a high-fat diet.

		Non-Tg		3xTgAD	
Male		Control	High-fat	Control	High-fat
7 weeks	Fat (g)	0.60±0.08	1.82±0.08^{c}	0.48±0.07	1.01±0.07^{c,iii}
	Liver (g)	1.10±0.04	1.02±0.04	1.14±0.04	1.10±0.63
	Spleen (g)	0.06±0.01	0.06±0.01	0.10±0.01^{iii}	0.11±0.01^{iii}
12 months	Fat (g)	1.56±0.14	1.38±0.14	1.05±0.15	3.93±0.15^{c,iii}
	Liver (g)	1.34±0.11	2.83±0.11^{c}	1.51±0.11	1.88±0.11^{a}
	Spleen (g)	0.08±0.02	0.12±0.02	0.15±0.02^{ii}	0.18±0.02^{ii}

		Non-Tg		3xTgAD	
Female		Control	High-fat	Control	High-fat
7 weeks	Fat (g)	0.28±0.08	0.59±0.07^{b}	0.59±0.09	1.07±0.09^{b,ii}
	Liver (g)	0.83±0.03	0.82±0.03	0.70±0.04	0.71±0.04
	Spleen (g)	0.07±0.01	0.07±0.01	0.09±0.01^{i}	0.11±0.01^{iii}
12 months	Fat (g)	1.47±0.49	4.67±0.52^{c}	1.14±0.49	4.96±0.52^{c}
	Liver (g)	1.20±0.05	1.46±0.05^{a}	1.11±0.05	1.13±0.05^{iii}
	Spleen (g)	0.14±0.02	0.15±0.03	0.12±0.02	0.21±0.03^{a}

Groups of male and female Non-Tg control and 3xTgAD mice were kept on a control or high-fat diet from 8 weeks of age. Fat (gonadal), liver and spleen weight in g were measured after 7 weeks and in a separate group of mice after 12 months on diet. Data are mean ± SEM, n = 9-12/group. ^{a} $p < 0.05$, ^{b} $p < 0.01$, ^{c} $p < 0.001$ versus control diet of the same genotype. ^{i} $p < 0.05$, ^{ii} $p < 0.01$, ^{iii} $p < 0.001$ versus Non-Tg on the same diet. Two-way ANOVA with Bonferroni's post hoc analysis

High-fat diet leads to hyperinsulinemia in male Non-Tg mice after 7 weeks on diet and in both male and female Non-Tg mice after 12 months

No change in plasma glucose levels were observed after 7 weeks or 12 months high-fat feeding in either male or female Non-Tg or 3xTgAD mice. After 7 weeks of high-fat diet plasma insulin levels were significantly increased in male Non-Tg mice by 100%, but no differences were observed in female Non-Tg or male or female 3xTgAD mice (Table 2). After 12 months, a high-fat diet increased plasma insulin in both male and female Non-Tg mice by 185% and 100% respectively (Table 2), but no effect was observed in male or female 3xTgAD mice (Table 2).

High-fat diet had no impact on blood pressure in both male and female Non-Tg and 3xTgAD mice

After 7 weeks, 6 and 12 months on a control diet, male and female 3xTgAD mice had lower heart rate than Non-Tg control mice, even prior to diet (at 8 weeks of age, data not shown). A high-fat diet caused a transient increase in heart rate after 7 weeks in female 3xTgAD compared to control fed 3xTgAD mice (Table 3) but had no effect on heart rate in male or female Non-Tg mice. There was no effect of genotype or diet on systolic blood pressure at any time point assessed in either male or female mice (Table 3).

Table 2

Glucose and insulin levels in male and female Non-Tg control and 3xTgAD mice in response to a high-fat diet

Male		Non-Tg		3xTgAD	
		Control	High-fat	Control	High-fat
7 weeks	Glucose (mmol/l)	8.8±0.6	10.1±0.6	9.1±0.5	10.7±0.5
	Insulin (ng/ml)	0.3±0.1	0.6±0.1[a]	0.3±0.6	0.4±0.6
12 months	Glucose (mmol/l)	9.2±0.6	8.0±0.6	7.7±0.6	9.4±0.6
	Insulin (ng/ml)	0.7±0.2	2.0±0.2[b]	0.9±0.2	1.2±0.2[i]

Female		Non-Tg		3xTgAD	
		Control	High-fat	Control	High-fat
7 weeks	Glucose (mmol/l)	7.4±0.6	8.1±0.5	9.0±0.6	9.0±0.7
	Insulin (ng/ml)	0.7±0.2	0.6±0.1	1.0±0.2	1.0±0.2
12 months	Glucose (mmol/l)	9.0±0.7	9.4±0.8	8.3±0.7	10.1±0.8
	Insulin (ng/ml)	0.3±0.1	0.6±0.1[a]	0.2±0.1	0.4±0.1

Groups of male and female Non-Tg control and 3xTgAD mice were maintained on a control or high-fat diet from 8 weeks of age. Blood glucose (mmol/l) and plasma insulin (ng/ml) levels were measured after 7 weeks and in a separate group of mice after 12 months on diet. Data are mean ± SEM, n = 9-12/group. [a] $p < 0.05$, [b] $p < 0.01$ versus control diet of the same genotype. [i] $p < 0.05$ versus Non-Tg on the same diet. Two-way ANOVA with Bonferroni's post hoc analysis.

Table 3

Systolic blood pressure and heart rate of male and female Non-Tg control and 3xTgAD mice in response to a high-fat diet

Male		Non-Tg		3xTgAD	
		Control	High-fat	Control	High-fat
7 weeks	Systolic (mmHg)	111.0±3.7	106.6±3.9	115.4±3.7	109.7±3.9
	Heart rate (bpm)	542.9±20.7	597.5±21.8	404.6±20.7[iii]	442.3±21.8[iii]
6 months	Systolic (mmHg)	109.7±2.6	108.5±2.8	107.7±3.0	112.0±2.7
	Heart rate (bpm)	550.7±19.3	591.0±24.2	389.4±22.6[iii]	472.4±20.2[ii]
12 months	Systolic (mmHg)	107.0±2.3	112.1±2.3	110.9±2.9	112.9±2.4
	Heart rate (bpm)	595.8±23.0	598.4±23.0	480.5±29.7[i]	550.7±24.2

Female		Non-Tg		3xTgAD	
		Control	High-fat	Control	High-fat
7 weeks	Systolic (mmHg)	111.3±4.7	113.2±4.3	104.0±5.2	105.4±4.9
	Heart rate (bpm)	464.4±17.1	516.6±15.7	379.2±18.9[ii]	485.0±17.9[c]
6 months	Systolic (mmHg)	95.8±3.2	93.7±3.1	104.0±3.1	108.3±3.3[i]
	Heart rate (bpm)	577.3±22.9	612.5±22.9	498.9±22.9	553.9±24.3
12 months	Systolic (mmHg)	96.2±3.5	96.5±3.5	102.3±4.1	97.3±3.7
	Heart rate (bpm)	576.5±18.4	608.2±18.4	485.0±21.3[i]	567.3±19.7

Groups of male and female Non-Tg control and 3xTgAD mice were maintained on a control or a high-fat diet from 8 weeks of age. Systolic blood pressure (mmHg) and heart rate (bpm) were monitored after 7 weeks and in a separate group of mice after 6 and 12 months on diet. Data are mean ± SEM, n = 9-12/group. [c] $p < 0.001$ versus control diet of the same genotype. [i] $p < 0.05$, [ii] $p < 0.01$, [iii] $p < 0.001$ versus Non-Tg on the same diet. Two-way ANOVA with Bonferroni's post hoc analysis.

High-fat diet impaired memory in male Non-Tg mice after 7 weeks and in both male and female Non-Tg mice after 12 months

In male and female Non-Tg and 3xTgAD mice, behaviour was assessed after 7 weeks and in a separate group of mice after 6 and 12 months on diet. In the open-field test, the % time moving was reduced in both male and female control-fed 3xTgAD compared to control-fed Non-Tg mice at most time-points assessed (Figure 3A-F).

At 7 weeks and 6 months on diet, a high-fat diet partially reversed this reduction in movement in male 3xTgAD mice (Figure 3A, C). There was no effect of a high-fat diet on movement in female and male control Non-Tg mice at 7 weeks, and 6 or 12 months (Figure 3A-E), apart from a significant reduction in females after 12 months of diet (Figure 3F).

To evaluate recognition memory, smell and NOR tests were used. For both tests no difference in the exploration between the two identical smells or objects during phase 1 were observed in all groups of mice (data not shown). In the smell recognition test, there was no effect of a high-fat diet on memory after 7 weeks, and 6 or 12 months in male or female Non-Tg mice as all mice spent significantly more time exploring the novel smell (Figure 4A-F). At all time-points assessed male and female 3xTgAD mice on either a control or a high-fat diet showed impaired memory as no difference in exploration between the familiar and novel smell was observed (Figure 4A-F).

In the NOR test, all Non-Tg mice on a control diet had intact memory while a high-fat diet led to significant impairments in memory (Figure 5A-D). Negative effects of high-fat diet on memory were observed after 7 weeks in male Non-Tg mice (Figure 5A) but not until 6 months in female Non-Tg mice (Figure 5D). Male and female 3xTgAD mice on control or high-fat diet were unable to perform the test, showing no difference between time exploring the novel and the familiar object at any time-point (Figure 5A-D), except at 7 weeks where females on a high-fat diet spent significantly less time exploring the novel object (Figure 5B).

In the Y-maze spontaneous alternation test, 7 weeks or 6 months of high-fat diet had no effect on the % alternation in either male or female Non-Tg mice (Figure 6A-D), but reductions were observed in male and female Non-Tg mice after 12 months on diet, (Figure 6E, F). At 12 months memory deficits were also observed in control fed female 3xTgAD versus Non-Tg mice (Figure 6F). No

memory impairments were seen in female control-fed 3xTgAD mice at 7 weeks or 6 months (Figure 6B, D) or at any point assessed in male control-fed 3xTgAD mice. (Figure 6A, C, E). A high-fat diet had no effect on memory in both male and female 3xTgAD mice at any time assessed (Figure 6A-F).

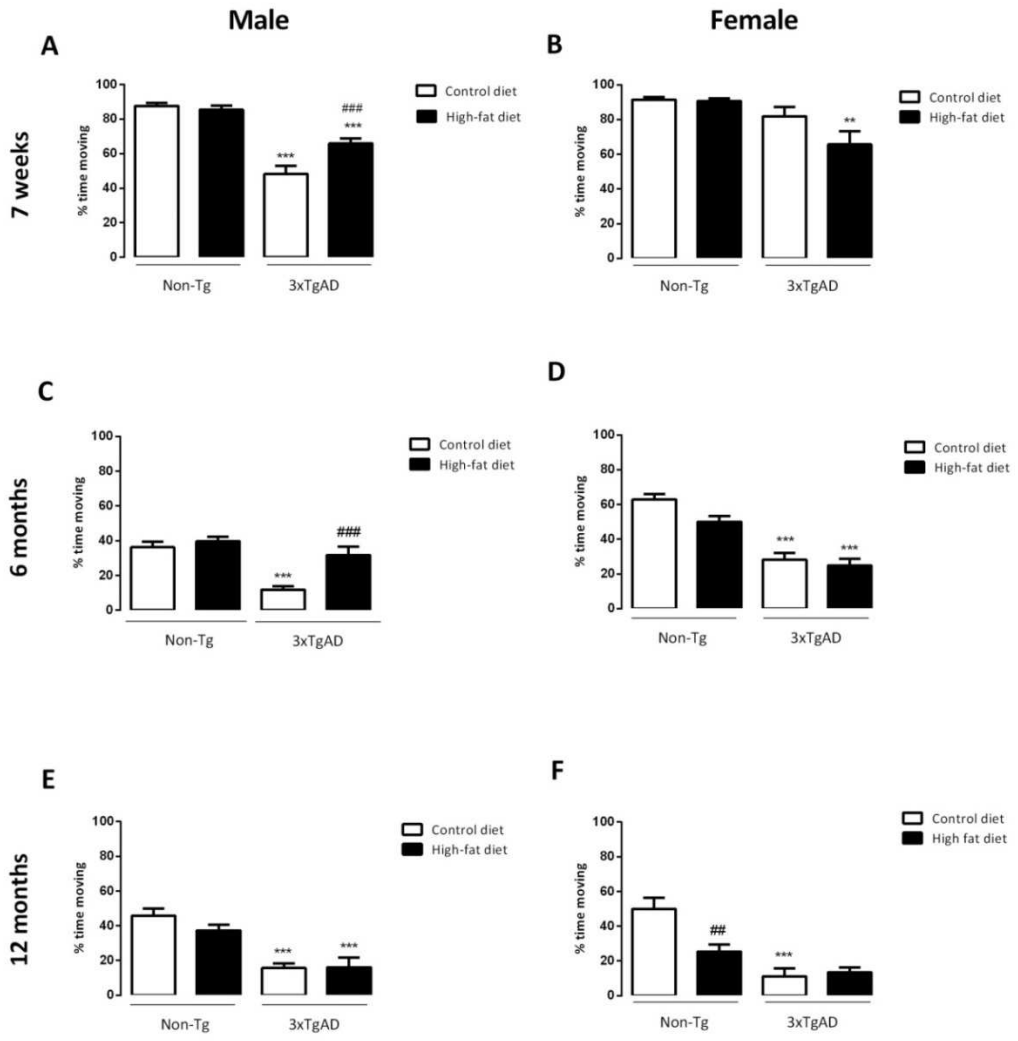


Figure 3. 3xTgAD mice show reduced movement in the open-field test. Groups of male and female Non-Tg control and 3xTgAD mice were maintained on a control or high-fat diet from 8 weeks of age and spontaneous exploratory behaviour was assessed by open-field after 7 weeks, and in a separate group of mice after 6 and 12 months on diet. Data are mean ± SEM, n = 9-12/group. ## $p < 0.01$, ### $p < 0.001$ versus control diet of the same genotype. ** $p < 0.01$, *** $p < 0.001$ versus Non-Tg on the same diet. Two-way ANOVA with Bonferroni's post hoc analysis.

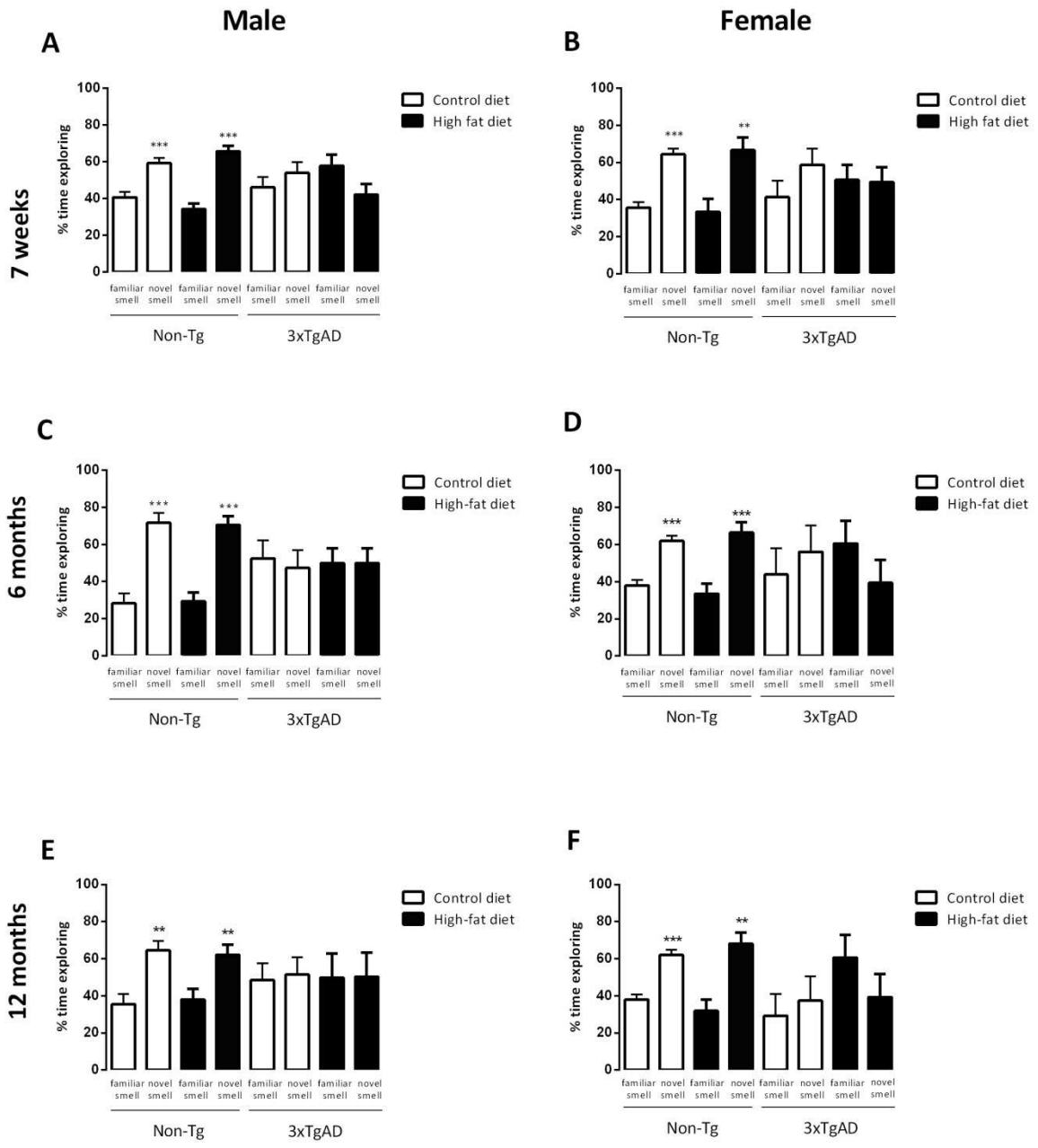


Figure 4. A high-fat diet has no effect in male and female mice when assessed with the smell recognition test. Separate groups of male and female Non-Tg control and 3xTgAD mice were maintained on a control or high-fat diet from 8 weeks of age. Memory was assessed by the smell recognition test after 7 weeks, and in a separate group of mice after 6 and 12 months on diet. A high-fat diet had no effect on memory in both male and female Non-Tg mice at all time points. Memory was impaired in control and high-fat fed 3xTgAD mice at all time points assessed. Data are mean ± SEM, n = 9-12/group. ** $p < 0.01$, *** $p < 0.001$ versus familiar smell. Student's *t* test.

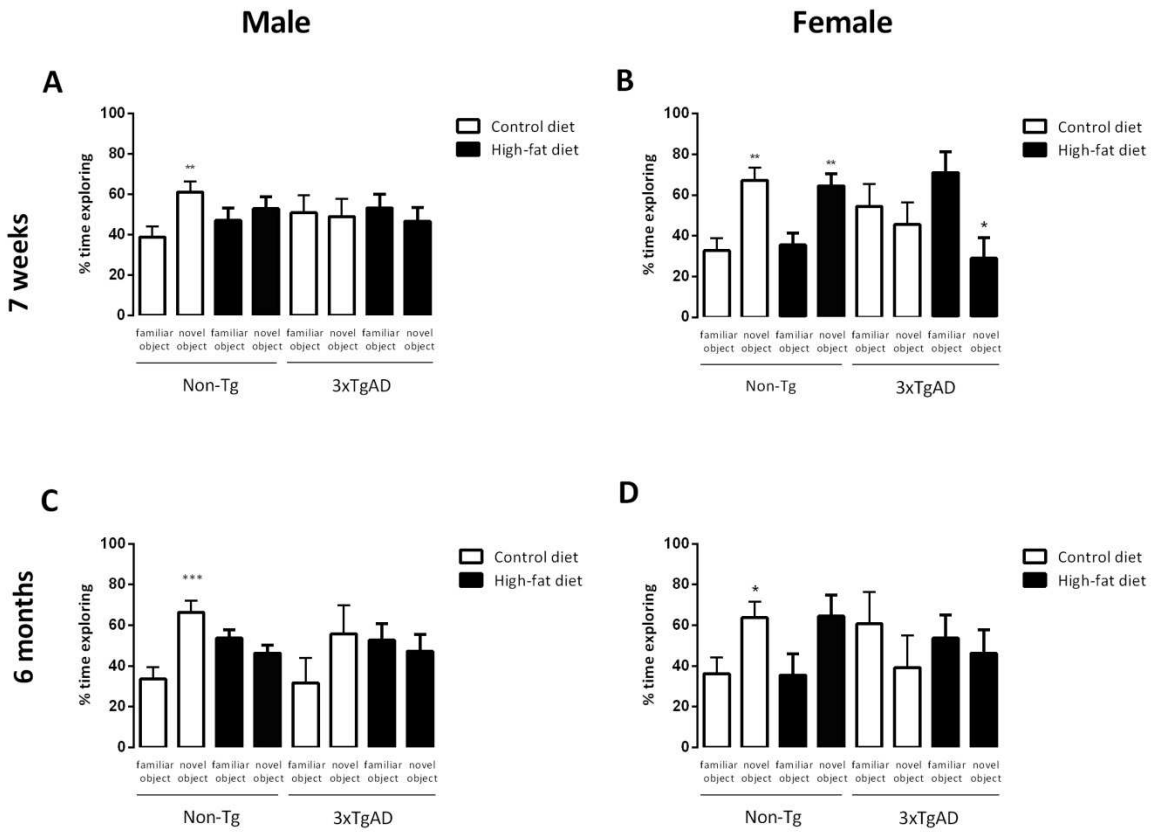


Figure 5. High-fat diet impairs memory earlier in male Non-Tg control mice in the novel object recognition test. Separate groups of male and female Non-Tg control and 3xTgAD mice were maintained on a control or high-fat diet from 8 weeks of age. Memory was assessed by the novel recognition test after 7 weeks and in a separate group of mice after 6 and 12 months on diet. In male Non-Tg mice, a high-fat diet impaired memory at 7 weeks but a significant effect on memory was not seen in Non-Tg female mice until 6 months of high-fat feeding. At 7 weeks and 6 months on control or high-fat diet both female and male 3xTgAD mice had memory deficits. Data are mean ± SEM, n = 9-12/group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ versus familiar object. Student's *t* test.

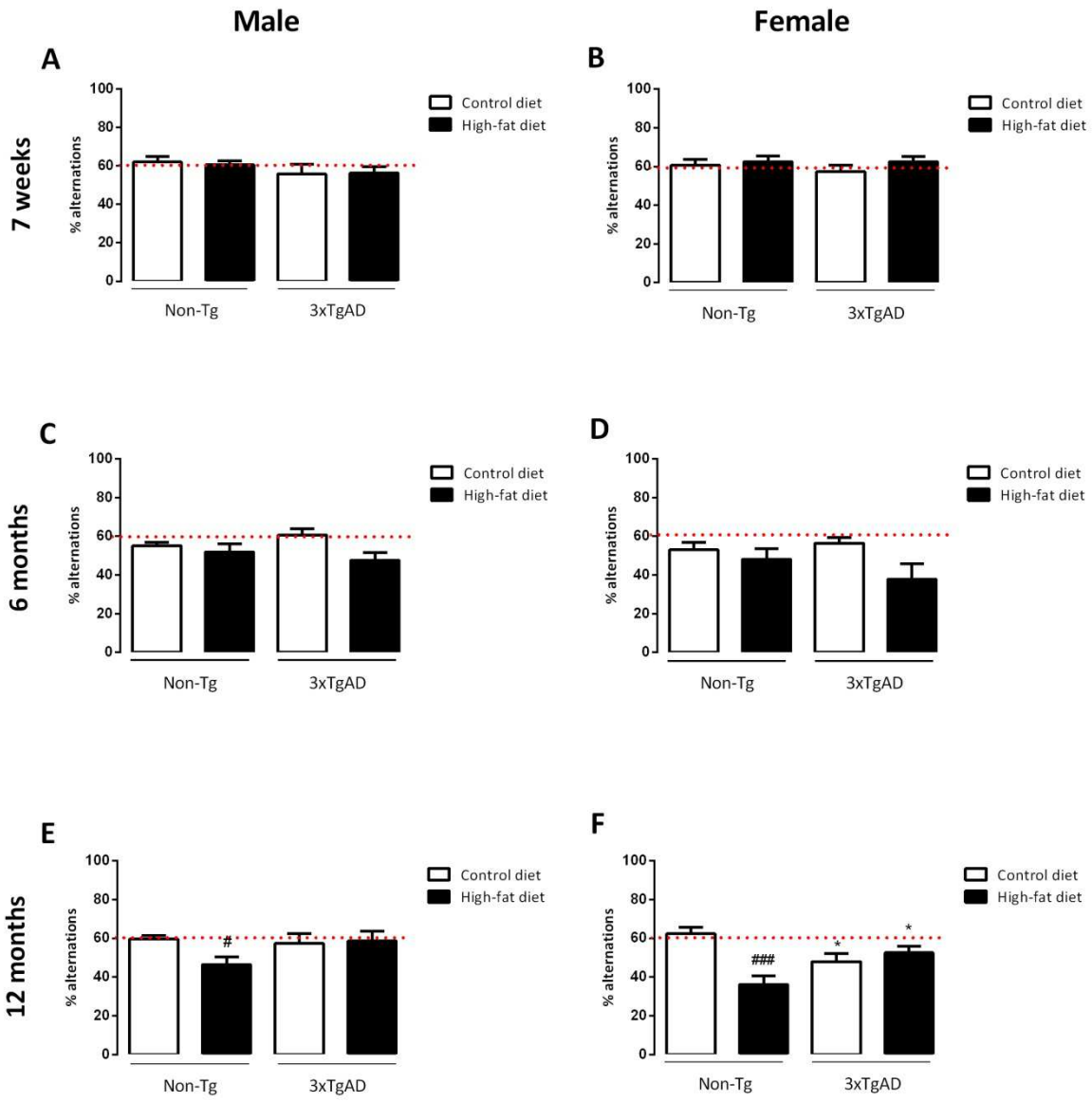


Figure 6. High-fat diet impairs memory in male and female Non-Tg control mice in the Y-maze spontaneous alternation test. Groups of male and female Non-Tg and 3xTgAD mice were maintained on a control or high-fat diet from 8 weeks of age. Memory was assessed by the Y-maze spontaneous alternation test after 7 weeks, and in a separate group of mice, after 6 and 12 months on diet. After 12 months a high-fat diet impaired memory in male and female Non-Tg control mice. Female 3xTgAD mice fed a control or high-fat diet had memory deficits after 12 months on diet. Data are mean ± SEM, n = 9-12/group. # $p < 0.05$, ### $p < 0.001$ versus control diet of the same genotype. * $p < 0.05$ versus Non-Tg on the same diet. Two-way ANOVA with Bonferroni's post hoc analysis.

Discussion

The present study is the first to compare the effects of a high-fat diet on memory using a battery of behavioural tests in both male and female mice, and to test these effects in both cognitively-normal (Non-Tg) animals and in a cognitively impaired mouse model of AD.

Our results showed that high-fat diet affected memory in control Non-Tg male and female mice with males being more susceptible as deficits were detected earlier in the NOR test. These data are consistent with a previous study showing that the different metabolic response to a high-fat diet in male mice results in poorer learning performance and greater deficits than females [43]. Deficits in memory were apparent earlier in Non-Tg males in the NOR test, suggesting that high-fat diet affects hippocampal-dependent memory, as seen previously [22-24, 33]. In addition, this result also demonstrates the importance of sex hormones to behaviour performance, as females perform better than males probably due to the role of estrogen in memory and learning mechanisms. This finding also suggest that the NOR test is more sensitive to early changes in memory induced by a high-fat diet than the Y-maze test in the present study. Open-field data demonstrated that a high-fat diet also had an effect on spontaneous exploratory behaviour in female but not male Non-Tg control mice.

A high-fat diet in 3xTgAD mice has previously been shown to worsen memory in both male [33, 34, 51] and female mice [34], results that were not confirmed here. However, using the smell recognition and novel object tests it was not possible to assess the effects of high-fat diet on memory in 3xTgAD animals, as these mice already had a memory deficit and therefore further changes could not be seen due to ceiling effect of the test. Although our previous study has shown effects of a high-fat diet on memory in 3xTgAD mice [33], memory impairments were only seen when diet was given for longer than 12 months when assessed by other tests, such as the Y-maze [33]. Here we saw no effect of a high-fat diet for 12 months on memory in male and female 3xTgAD mice when assessed by Y-maze test. However, we do observe that control-fed females perform worse than male 3xTgAD mice. These results are in line with previous studies demonstrating higher impairment in female 3xTgAD when compared with male 3xTgAD mice [52, 53], which might be due to their exacerbated AD pathology [54]. In the open-field test both male and female 3xTgAD mice

exhibited reduced spontaneous exploratory activity, as demonstrated previously [55, 56], which could suggest an increase in anxiety levels probably due to its genetic background (tau mutation) and ultimately affect performance in learning tests such as NOR and smell recognition. Although we do observe changes in female and male 3xTgAD mice in both memory and general behaviour, pathology was not assessed in the present study, and so we are not able to conclude if such changes in behaviour are associated with increased amyloid and/or tau that could be gender dependent. However, a previous study has shown that changes in behaviour, and the negative effect of high-fat diet, were not associated with increased pathology in 3xTgAD mice [33].

A number of differences in the vulnerability of male and female rodents to diet-induced obesity have been reported, with conflicting results. While some have shown that male mice are more susceptible to diet-induced obesity and associated metabolic alterations [41-43, 47, 48], others demonstrate that females gain more body weight and adipose tissue [41, 44]. In other studies it has been also demonstrated that females show better insulin sensitivity [42, 44, 45, 47] and less immune cell infiltration in adipose tissue on a high-fat feeding [41, 44]. Here, a high-fat diet was associated with a greater increase in body weight gain, adipose tissue accumulation and plasma insulin levels in male Non-Tg mice after short-term diet. At this time only male Non-Tg mice showed memory deficits in response to a high-fat diet. After 12 months on a high-fat diet, when memory deficits are present in Non-Tg mice of both sexes, insulin was increased in both sexes, yet with a greater increase in male Non-Tg mice. Although we do not observe altered glucose levels with high-fat diet, insulin levels were increased in both male and female Non-Tg mice fed on a high-fat diet at 12 months, but only in male Non-Tg mice at 7 weeks, suggesting that females have better insulin sensitivity as seen in other studies [44, 47], but only during a short time on diet. Hyperinsulinemia has been associated with high-fat diets due to increase of intra-abdominal fat deposits [57, 58]. It is known that high peripheral levels of insulin can lead to insulin resistance [59-63], which has been associated with impaired memory [29, 59, 64-66] and brain insulin resistance in AD [67-70]. In fact, ovarian hormones have been shown to protect female mice against insulin resistance, known in humans as metabolically benign obesity, where there is an increase in visceral and total body fat content without a rise in insulin levels [71, 72]. Insulin can play an important role in hippocampal-

dependent memory processes [66, 73] and the hippocampus shows high levels of insulin receptor expression [74]. It is possible therefore that deficits in hippocampal dependent memory observed here may arise from peripheral insulin resistance/reduced insulin sensitivity induced by high-fat diet.

One reason why 3xTgAD mice were less affected by a high-fat diet than Non-Tg controls in the Y-maze test could be due to the different metabolic response to the diet in 3xTgAD mice. In contrast to Non-Tg control mice, a high-fat diet had no effect on insulin levels in male and female 3xTgAD, suggesting that these animals have better insulin sensitivity than Non-Tg controls. Insulin resistance has been correlated with AD and memory impairment [59, 64, 74, 75]. Insulin appears to have an important role in the brain and studies have shown it can be neuroprotective [36, 76, 77] and modulate synaptic plasticity [78]. Although we do not have data to conclude about insulin resistance, better insulin sensitivity could be one reason why the high-fat diet did not affect memory in 3xTgAD mice in those tests where effects of diet could be determined.

Adipose tissue is a major endocrine organ that regulates energy balance, glucose homeostasis, insulin sensitivity and inflammation, by producing cytokines and adipokines. An increase of adipose tissue has been associated with a greater infiltration and activation of immune cells, which leads to systemic inflammation, a feature known to occur in obesity [79, 80]. Indeed, inflammatory cytokines produced by adipose tissue are thought to contribute to cognitive deficits in obesity, as removal of adipose tissue, through lipectomy, can prevent inflammation, cognitive dysfunction and alternations in synaptic function in a genetic mouse model of obesity [30]. However, the inflammatory state of our animals was not assessed so it is impossible to conclude if inflammation could be involved in memory deficits seen in this study. Moreover, recent studies have demonstrated that adipose tissue is able to generate amyloid-β (Aβ) and that amyloid precursor protein (APP) is upregulated in obese patients [81], suggesting that memory deficits could result from increase adipose tissue producing high levels of peripheral Aβ that could ultimately infiltrate in the brain.

We do observe that with a high-fat diet there is an increase in gonadal fat content in both male and female Non-Tg mice after 7 weeks, but only in females after 12 months, which suggest that in males adipose tissue could be deposited in other visceral organs and subcutaneous compartments after long-term high-fat feeding. One of the possible organs accumulating fat after long-term diet

could be the liver. Male and female Non-Tg mice fed on a high-fat diet for 12 months presented enlarged livers that were paler and had fat droplets. Such phenotype could result from increased inflammation or non-alcoholic fatty liver disease (NAFLD), a common disorder in obesity that is also associated with insulin resistance [82, 83]. Male Non-Tg mice had greater increase in liver weight than females and also present greater levels of insulin.

The association between obesity and hypertension is well known [14]. In addition, hypertension has also been considered a risk factor for AD [84] and have been implicated in impaired cognitive function in healthy individuals [85-87], therefore blood pressure was measured in the present study. A high-fat diet had no effect on systolic blood pressure in either male or female Non-Tg and 3xTgAD mice and it is therefore unlikely that changes in blood pressure were responsible for the negative effects of a high-fat diet on memory seen here.

In summary, we demonstrate for the first time in the same study the effect of a high-fat diet on memory in both male and female Non-Tg control mice and in mouse model of AD (3xTgAD). A high-fat diet affected memory in both male and female Non-Tg control mice, although male Non-Tg controls were more susceptible to diet-induced obesity over the short-term compared to female mice. After short-term diet, male Non-Tg control mice gained more body weight and adipose tissue, which was associated with hyperinsulinemia and memory deficits. Long-term diet affected memory in both male and female Non-Tg mice, which was associated with increased levels of insulin, yet greater in male mice than females. These different metabolic outcomes to a high-fat diet, in particular insulin, might therefore be important in explaining why male and female control mice show differences in the effect of a high-fat diet on memory. Future work should address these sex-dependent metabolic changes induced by high-fat diet in order to better understand its influence in obesity and memory.

References

1. Elias, M.F., et al., *Lower cognitive function in the presence of obesity and hypertension: the Framingham heart study.* Int J Obes Relat Metab Disord, 2003. **27**(2): p. 260-268.
2. Elias, M.F., et al., *Obesity, diabetes and cognitive deficit: The Framingham Heart Study.* Neurobiology of Aging, 2005. **26**(1, Supplement): p. 11-16.
3. Waldstein, S.R. and L.I. Katzel, *Interactive relations of central versus total obesity and blood pressure to cognitive function.* Int J Obes Relat Metab Disord, 2005. **30**(1): p. 201-207.
4. Bruce-Keller, A.J., J.N. Keller, and C.D. Morrison, *Obesity and vulnerability of the CNS.* Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease, 2009. **1792**(5): p. 395-400.
5. Anstey, K.J., et al., *The influence of smoking, sedentary lifestyle and obesity on cognitive impairment-free life expectancy.* International Journal of Epidemiology, 2014.
6. Kanoski, S.E. and T.L. Davidson, *Western diet consumption and cognitive impairment: Links to hippocampal dysfunction and obesity.* Physiology & Behavior, 2011. **103**(1): p. 59-68.
7. Xu, W.L., et al., *Midlife overweight and obesity increase late-life dementia risk: a population-based twin study. .* Neurology, 2011. **76**: p.:1568–1574.
8. Fitzpatrick, A.L., et al., *Midlife and late-life obesity and the risk of dementia: Cardiovascular health study.* Archives of Neurology, 2009. **66**(3): p. 336-342.
9. Hassing, L.B., et al., *Overweight in midlife and risk of dementia: a 40-year follow-up study.* Int J Obes, 2009. **33**(8): p. 893-898.
10. Whitmer, R.A., et al., *Obesity in middle age and future risk of dementia: a 27 year longitudinal population based study*. Vol. 330. 2005. 1360.
11. Whitmer, R.A., et al., *Central obesity and increased risk of dementia more than three decades later.* Neurology, 2008. **71**(14): p. 1057-64.
12. Beydoun, M.A., et al., *Association of Adiposity Status and Changes in Early to Mid-Adulthood With Incidence of Alzheimer's Disease.* American Journal of Epidemiology, 2008. **168**(10): p. 1179-1189.
13. Gustafson, D.R., *Adiposity and cognitive decline: underlying mechanisms.* Alzheimer's Disease, 2012. **30**: p. S97–112.
14. Naderali, E.K., S.H. Ratcliffe, and M.C. Dale, *Review: Obesity and Alzheimer's Disease: A Link Between Body Weight and Cognitive Function in Old Age.* American Journal of Alzheimer's Disease and Other Dementias, 2009. **24**(6): p. 445-449.
15. Cornier, M.-A., et al., *The Metabolic Syndrome.* Endocrine Reviews, 2008. **29**(7): p. 777-822.
16. Panza, F., et al., *Metabolic Syndrome and Cognitive Impairment: Current Epidemiology and Possible Underlying Mechanisms.* Journal of Alzheimer's Disease, 2010. **21**(3): p. 691-724.
17. Frisardi, V., et al., *Metabolic-cognitive syndrome: A cross-talk between metabolic syndrome and Alzheimer's disease.* Ageing Research Reviews, 2010. **9**(4): p. 399-417.
18. Misiak, B., J. Leszek, and A. Kiejna, *Metabolic syndrome, mild cognitive impairment and Alzheimer's disease—The emerging role of systemic low-grade inflammation and adiposity.* Brain research bulletin, 2012. **89**(3–4): p. 144-149.
19. Mueller, K., et al., *Overweight and obesity are associated with neuronal injury in the human cerebellum and hippocampus in young adults: a combined MRI, serum marker and gene expression study.* Transl Psychiatry, 2012. **2**: p. e200.
20. Raji, C.A., et al., *Brain structure and obesity.* Human Brain Mapping, 2010. **31**(3): p. 353-364.
21. Tucsek, Z., et al., *Aging Exacerbates Obesity-induced Cerebromicrovascular Rarefaction, Neurovascular Uncoupling, and Cognitive Decline in Mice.* The Journals of Gerontology Series A: Biological Sciences and Medical Sciences, 2014.
22. Boitard, C., et al., *Impairment of hippocampal-dependent memory induced by juvenile high-fat diet intake is associated with enhanced hippocampal inflammation in rats.* Brain, Behavior, and Immunity, 2014. **40**(0): p. 9-17.
23. Valladolid-Acebes, I., et al., *High-fat diets impair spatial learning in the radial-arm maze in mice.* Neurobiology of Learning and Memory, 2011. **95**(1): p. 80-85.

24. Valladolid-Acebes, I., et al., *Spatial memory impairment and changes in hippocampal morphology are triggered by high-fat diets in adolescent mice. Is there a role of leptin?* Neurobiology of Learning and Memory, 2013. **106**(0): p. 18-25.
25. Heyward, F.D., et al., *Adult mice maintained on a high-fat diet exhibit object location memory deficits and reduced hippocampal SIRT1 gene expression.* Neurobiology of Learning and Memory, 2012. **98**(1): p. 25-32.
26. Tucker, K.R., et al., *Olfactory ability and object memory in three mouse models of varying body weight, metabolic hormones, and adiposity.* Physiology & Behavior, 2012. **107**(3): p. 424-432.
27. Reichelt, A.C., et al., *Dietary-induced obesity disrupts trace fear conditioning and decreases hippocampal reelin expression.* Brain, Behavior, and Immunity, 2015. **43**: p. 68-75.
28. Sobesky, J.L., et al., *High-fat diet consumption disrupts memory and primes elevations in hippocampal IL-1β, an effect that can be prevented with dietary reversal or IL-1 receptor antagonism.* Brain, Behavior, and Immunity, 2014. **42**: p. 22-32.
29. Arnold, S.E., et al., *High fat diet produces brain insulin resistance, synaptodendritic abnormalities and altered behavior in mice.* Neurobiology of Disease, 2014. **67**(0): p. 79-87.
30. Erion, J.R., et al., *Obesity Elicits Interleukin 1-Mediated Deficits in Hippocampal Synaptic Plasticity.* The Journal of Neuroscience, 2014. **34**(7): p. 2618-2631.
31. Davidson, T.L., et al., *Contributions of the hippocampus and medial prefrontal cortex to energy and body weight regulation.* Hippocampus, 2009. **19**(3): p. 235-252.
32. Davidson, T.L., et al., *Hippocampal Lesions Impair Retention of Discriminative Responding Based on Energy State Cues.* Behavioral neuroscience, 2010. **124**(1): p. 97-105.
33. 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.* Neurobiology of Aging, 2014. **35**(8): p. 1821-1832.
34. Barron, A.M., et al., *Sex-Specific Effects of High Fat Diet on Indices of Metabolic Syndrome in 3xTg-AD Mice: Implications for Alzheimer's Disease.* PLoS ONE, 2013. **8**(10): p. e78554.
35. Takalo, M., et al., *High-fat diet increases tau expression in the brain of T2DM and AD mice independently of peripheral metabolic status.* The Journal of Nutritional Biochemistry, 2014. **25**(6): p. 634-641.
36. Vandal, M., et al., *Insulin Reverses the High-Fat Diet–Induced Increase in Brain Aβ and Improves Memory in an Animal Model of Alzheimer Disease.* Diabetes, 2014.
37. Ramos-Rodriguez, J.J., et al., *Prediabetes-induced vascular alterations exacerbate central pathology in APPswe/PS1dE9 mice.* Psychoneuroendocrinology, 2014. **48**(0): p. 123-135.
38. Maesako, M., et al., *Environmental enrichment ameliorated high-fat diet-induced Aβ deposition and memory deficit in APP transgenic mice.* Neurobiology of Aging, 2012. **33**(5): p. 1011.e11-1011.e23.
39. Power, M.L. and J. Schulkin, *Sex differences in fat storage, fat metabolism, and the health risks from obesity: possible evolutionary origins.* British Journal of Nutrition, 2008. **99**(05): p. 931-940.
40. Jamshed, N., et al., *Alzheimer disease in post-menopausal women: Intervene in the critical window period.* Journal of Mid-Life Health, 2014. **5**(1): p. 38-40.
41. Nickelson, K.J., et al., *A Comparison of Inflammatory and Oxidative Stress Markers in Adipose Tissue from Weight-Matched Obese Male and Female Mice.* Experimental Diabetes Research, 2012. **2012**: p. 8.
42. Pettersson, U.S., et al., *Female Mice are Protected against High-Fat Diet Induced Metabolic Syndrome and Increase the Regulatory T Cell Population in Adipose Tissue.* PLoS ONE, 2012. **7**(9): p. e46057.
43. Hwang, L.-L., et al., *Sex Differences in High-fat Diet-induced Obesity, Metabolic Alterations and Learning, and Synaptic Plasticity Deficits in Mice.* Obesity, 2010. **18**(3): p. 463-469.
44. Medrikova, D., et al., *Sex differences during the course of diet-induced obesity in mice: adipose tissue expandability and glycemic control.* Int J Obes, 2012. **36**(2): p. 262-272.
45. Naville, D., et al., *Low-dose food contaminants trigger sex-specific, hepatic metabolic changes in the progeny of obese mice.* The FASEB Journal, 2013. **27**(9): p. 3860-3870.

46. Lorbek, G., et al., *Sex Differences in the Hepatic Cholesterol Sensing Mechanisms in Mice.* Molecules, 2013. **18**(9): p. 11067-11085.
47. Senthil Kumar, S.P.D., et al., *Distinct metabolic effects following short-term exposure of different high-fat diets in male and female mice.* Endocrine Journal, 2014. **61**(5): p. 457-470.
48. Yang, Y., et al., *Variations in body weight, food intake, and body composition after long-term high-fat diet feeding in C57BL/6J mice.* Obesity, 2014: p. n/a-n/a.
49. 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 - 421.
50. Ennaceur, A. and J. Delacour, *A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data.* Behavioural Brain Research, 1988. **31**(1): p. 47-59.
51. Vandal, M., et al., *Insulin Reverses the High-Fat Diet–Induced Increase in Brain Aß and Improves Memory in an Animal Model of Alzheimer Disease.* Diabetes, 2014. **63**(12): p. 4291-4301.
52. Blázquez, G., et al., *Cognitive and emotional profiles of aged Alzheimer's disease (3xTgAD) mice: Effects of environmental enrichment and sexual dimorphism.* Behavioural Brain Research, 2014. **268**(0): p. 185-201.
53. Clinton, L.K., et al., *Age-dependent sexual dimorphism in cognition and stress response in the 3xTg-AD mice.* Neurobiology of Disease, 2007. **28**(1): p. 76-82.
54. Hirata-Fukae, C., et al., *Females exhibit more extensive amyloid, but not tau, pathology in an Alzheimer transgenic model.* Brain Research, 2008. **1216**(0): p. 92-103.
55. Nelson, R.L., et al., *Prophylactic treatment with paroxetine ameliorates behavioral deficits and retards the development of amyloid and tau pathologies in 3xTgAD mice.* Experimental neurology, 2007. **205**(1): p. 166-176.
56. Halagappa, V.K.M., et al., *Intermittent fasting and caloric restriction ameliorate age-related behavioral deficits in the triple-transgenic mouse model of Alzheimer's disease.* Neurobiology of Disease, 2007. **26**(1): p. 212-220.
57. Fernandez, M.L., *The Metabolic Syndrome.* Nutrition Reviews, 2007. **65**: p. S30-S34.
58. Michalakis, K., et al., *The complex interaction between obesity, metabolic syndrome and reproductive axis: A narrative review.* Metabolism, 2013. **62**(4): p. 457-478.
59. Stranahan, A.M., et al., *Diet-induced insulin resistance impairs hippocampal synaptic plasticity and cognition in middle-aged rats.* Hippocampus, 2008. **18**(11): p. 1085-1088.
60. Chudnovskiy, R., et al., *Consumption of Clarified Grapefruit Juice Ameliorates High-Fat Diet Induced Insulin Resistance and Weight Gain in Mice.* PLoS ONE, 2014. **9**(10): p. e108408.
61. Feng, X.-T., et al., *Pollen Typhae total flavone improves insulin resistance in high-fat diet and low-dose streptozotocin-induced type 2 diabetic rats.* Bioscience, Biotechnology, and Biochemistry, 2014. **78**(10): p. 1738-1742.
62. Kodama, K., et al., *Anti-CD44 Antibody Treatment Lowers Hyperglycemia and Improves Insulin Resistance, Adipose Inflammation, and Hepatic Steatosis in Diet-Induced Obese Mice.* Diabetes, 2014.
63. Ma, Y., M. Gao, and D. Liu, *Chlorogenic Acid Improves High Fat Diet-Induced Hepatic Steatosis and Insulin Resistance in Mice.* Pharmaceutical Research, 2014: p. 1-10.
64. Wu, H.-W., et al., *A high-fructose diet induces hippocampal insulin resistance and exacerbates memory deficits in male Sprague-Dawley rats.* Nutritional Neuroscience, 2014. **0**(0): p. null.
65. McNeilly, A.D., et al., *High fat feeding promotes simultaneous decline in insulin sensitivity and cognitive performance in a delayed matching and non-matching to position task.* Behavioural Brain Research, 2011. **217**(1): p. 134-141.
66. McNay, E.C., et al., *Hippocampal memory processes are modulated by insulin and high-fat-induced insulin resistance.* Neurobiology of Learning and Memory, 2010. **93**(4): p. 546-553.
67. Hoyer, S., *The aging brain. Changes in the neuronal insulin/insulin receptor signal transduction cascade trigger late-onset sporadic Alzheimer disease (SAD). A mini-review.* Journal of Neural Transmission, 2002. **109**(7-8): p. 991-1002.

68. Rivera, E.J., et al., *Insulin and insulin-like growth factor expression and function deteriorate with progression of Alzheimer's disease: Link to brain reductions in acetylcholine.* Journal of Alzheimer's Disease, 2005. **8**(3): p. 247-268.
69. Talbot, K., et al., *Demonstrated brain insulin resistance in Alzheimer's disease patients is associated with IGF-1 resistance, IRS-1 dysregulation, and cognitive decline.* The Journal of Clinical Investigation, 2012. **122**(4): p. 1316-1338.
70. Cholerton, B., L.D. Baker, and S. Craft, *Insulin, cognition, and dementia.* European Journal of Pharmacology, 2013. **719**(1–3): p. 170-179.
71. Stefan, N., et al., *Identification and characterization of metabolically benign obesity in humans.* Archives of Internal Medicine, 2008. **168**(15): p. 1609-1616.
72. Wildman, R.P., et al., *The obese without cardiometabolic risk factor clustering and the normal weight with cardiometabolic risk factor clustering: Prevalence and correlates of 2 phenotypes among the us population (nhanes 1999-2004).* Archives of Internal Medicine, 2008. **168**(15): p. 1617-1624.
73. Park, C.R., *Cognitive effects of insulin in the central nervous system.* Neuroscience & Biobehavioral Reviews, 2001. **25**(4): p. 311-323.
74. De Felice, F.G. and S.T. Ferreira, *Inflammation, Defective Insulin Signaling, and Mitochondrial Dysfunction as Common Molecular Denominators Connecting Type 2 Diabetes to Alzheimer Disease.* Diabetes, 2014. **63**(7): p. 2262-2272.
75. Craft, S., *Insulin resistance syndrome and Alzheimer's disease: Age- and obesity-related effects on memory, amyloid, and inflammation.* Neurobiology of Aging, 2005. **26**(1, Supplement): p. 65-69.
76. Bomfim, T.R., et al., *An anti-diabetes agent protects the mouse brain from defective insulin signaling caused by Alzheimer's disease–associated Aβ oligomers.* The Journal of Clinical Investigation, 2012. **122**(4): p. 1339-1353.
77. Plum, L., M. Schubert, and J.C. Brüning, *The role of insulin receptor signaling in the brain.* Trends in Endocrinology & Metabolism, 2005. **16**(2): p. 59-65.
78. Wan, Q., et al., *Recruitment of functional GABAA receptors to postsynaptic domains by insulin.* Nature, 1997. **388**(6643): p. 686-690.
79. Strissel, K.J., et al., *Adipocyte Death, Adipose Tissue Remodeling, and Obesity Complications.* Diabetes, 2007. **56**(12): p. 2910-2918.
80. Lumeng, C.N., et al., *Increased Inflammatory Properties of Adipose Tissue Macrophages Recruited During Diet-Induced Obesity.* Diabetes, 2007. **56**(1): p. 16-23.
81. Lee, Y.-H., et al., *Amyloid Precursor Protein Expression Is Upregulated in Adipocytes in Obesity.* Obesity, 2008. **16**(7): p. 1493-1500.
82. El-Karaksy, H.M., et al., *The value of different insulin resistance indices in assessment of non-alcoholic fatty liver disease in overweight/obese children.* Diabetes & Metabolic Syndrome: Clinical Research & Reviews, (0).
83. Utzschneider, K.M. and S.E. Kahn, *The Role of Insulin Resistance in Nonalcoholic Fatty Liver Disease.* The Journal of Clinical Endocrinology & Metabolism, 2006. **91**(12): p. 4753-4761.
84. Kivipelto, M., et al., *Obesity and vascular risk factors at midlife and the risk of dementia and alzheimer disease.* Archives of Neurology, 2005. **62**(10): p. 1556-1560.
85. Kovács, K.R., et al., *Elevated LDL-C combined with hypertension worsens subclinical vascular impairment and cognitive function.* Journal of the American Society of Hypertension, 2014. **8**(8): p. 550-560.
86. Vicario, A., et al., *Hypertension and Cognitive Decline: Impact on Executive Function.* The Journal of Clinical Hypertension, 2005. **7**(10): p. 598-604.
87. Papademetriou, V., *Hypertension and cognitive function. Blood pressure regulation and cognitive function: a review of the literature.* Geriatrics, 2005. **60**(1): p. 20-22.

Supplemental Material

Earlier memory impairment after a high-fat diet in male versus female mice is associated with hyperinsulinemia

Isaura Martins, Stuart M. Allan, Catherine B. Lawrence

The supplementary data presented in this section correspond to extra behaviour analysis of open-field and Y-maze spontaneous tests (Supplementary figure 4-6), analysis of hippocampal ICAM-1 levels in male and female Non-Tg and 3xTgAD mice after 7 weeks of diet (Supplementary figure 7) and western blot analysis of hippocampal tissue from only female Non-Tg and 3xTgAD mice after 12 months of diet (Supplementary figure 8). Due to lack of animal tissue from male Non-Tg and 3xTgAD cohort at 12 months of diet both ICAM-1 levels and western blot analysis could not be performed.

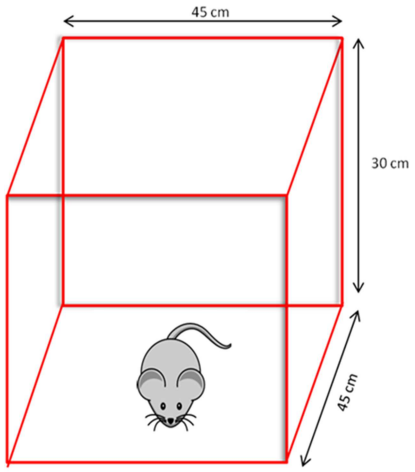
Supplementary Methods

Behaviour apparatus

Open-Field test

Open-field is a test that allows the measurement of exploratory activity by placing the mouse in an unfamiliar environment. Locomotor activity and anxiety-like behaviour can also be evaluated by observing the rearing behaviour of the mouse. The open-field arena consists of a square opaque Perspex box (45 cm x 45 cm x 30cm) (Supplementary Figure 1) that was placed on the floor of the behavioural testing room. The mouse was positioned in the centre of the arena in all trials and allowed to explore for 5 min.

The test was recorded live and transmitted to the laptop, which allowed observation of the total number of times the mice reared and the total number of defecations. These two parameters are also a measurement of exploration (rearing) and anxiety (defecation). The Any-Maze tracking software system (Stoelting Co, Dublin, Ireland) divided the arena into 16 equal cells and calculated the number of entries/cell. Total time moving was then measured and % time moving calculated.

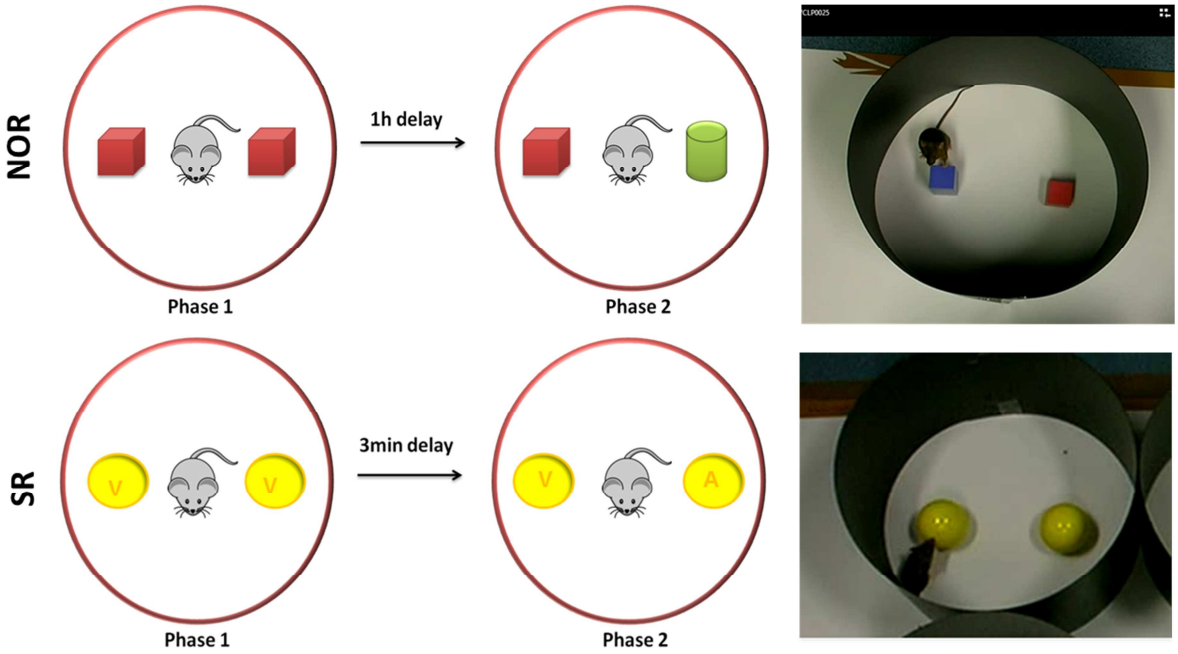


Supplementary Figure 1. Open-field test arena. In the open-field test the mouse was placed in the centre of the arena faced always to the same side and allowed to explore for 5 min. During the test the video camera was connected to a laptop with software that recorded the number of entries in each cell and the percentage of time moving within the arena. The number of defecations and rearing was manually recorded.

Smell and novel object recognition tests

The smell and NOR tests were used to evaluate non-associative working memory and recognition memory. The arena consisted of a black opaque polycarbonate circular arena (30cm) with no base (Supplementary Figure 2). For both tests the same arena was used and cleaned all times. Two days before the experiment the animals were allowed to explore a circular and empty arena for 5 min. On the day of the test, the mice were placed in the testing room 30 min prior to the task. In phase 1 two identical and unfamiliar smells/objects were placed in the centre of the arena at the same distance of each other and from the edges of the arena. The mouse was positioned in the centre of the arena and allowed to explore for 10 min the two smells/objects (phase 1). After the

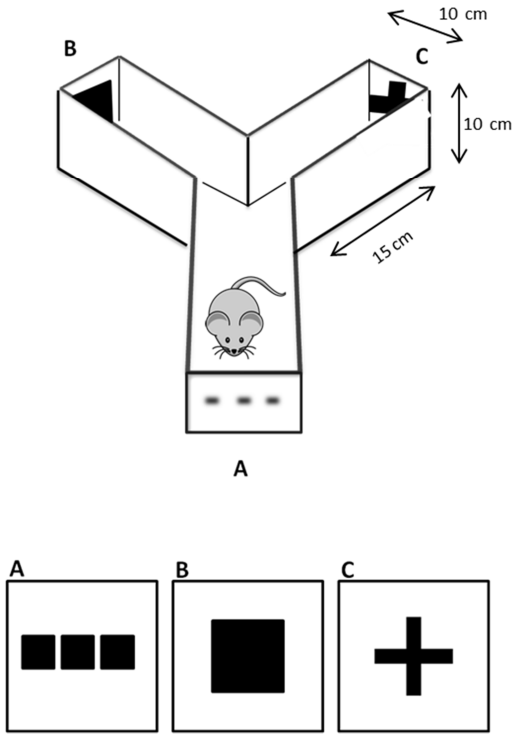
delay, 3 min for smell recognition (SR) and 1 h for NOR, one of the familiar smell/object was replaced for a new one and the animal is allowed to explore for 4 min (Supplementary Figure 2).



Supplementary Figure 2. The smell and novel object recognition apparatus. In both smell recognition (SR) and novel object recognition (NOR) phase 1 consisted in 10 min followed by a delay of 1 h for NOR and 3 min for smell recognition. Phase 2 consisted in 4 min for each test. In phase 1 both objects and smells (V, vanilla) are identical and in phase 2 one of the object and smell is replaced by a new one. V, vanilla; A; Almond.

Y-maze spontaneous alternation test

The Y-maze spontaneous alternation is a method to measure exploratory activity in mice to a new environment based on short-term memory (working memory) (Supplementary Figure 3). The maze (arm dimensions; 15 cm x 10 cm x 10 cm) was placed on the floor and the experimenter seated directly in front of the arm A. Each animal was placed facing the end of arm A and allowed to explore for 8 min. Spontaneous alternation was defined as three consequent entries in different arms in a random order, named triplets (eg, ABC; BCA; etc.). The percentage number of alternations between arms was calculated and defined as the number of alternations (triplets) divided by the total number of entries in the arms minus two ($\% \, alternations = \frac{no.triplets}{total\ no.entries-2}$).



Supplementary Figure 3. Y-maze spontaneous alternation maze. The mouse was placed faced to the A arm and allowed to explore the maze for 8 min. The images A, B and C were located in the ends of each arm of the maze to serve as cues to the mice.

Tissue collection and processing

At the end of the feeding period, mice were terminally anesthetized with 3.5% isoflurane (30% O_2 and 70% N_2O), blood was taken from the heart using 3.8% sodium citrate as an anticoagulant and plasma obtained after centrifugation was stored at -80°C until assay. To remove all blood, animals were perfused transcardially with 0.9% saline and the whole hippocampus was removed. Hippocampal samples then were homogenised using a mechanical homogeniser (IKA, USA) in tissue buffer (50 mmol/L Tris-HCl pH 7.4, 150 mmol/L NaCl, 5 mmol/L $CaCl_2$, 0.02% NaN_3, 1% Triton X) with added protease inhibitor cocktail 1 (Calbiochem, UK) and phosphatase inhibitors (1 mM sodium orthovanadate and 5 mM sodium fluoride), in ratio of 5 µl per milligram of tissue. Samples were incubated on ice for 30 min and then centrifuged at 15,000 *g* for 30 min at 4 ºC. Supernatants were collected and analysed for protein concentration using a bicinchoninic acid

(BCA) assay (Pierce, Thermo-Fisher Scientific, Northumberland, UK). Samples were stored frozen at -20 ºC until further use.

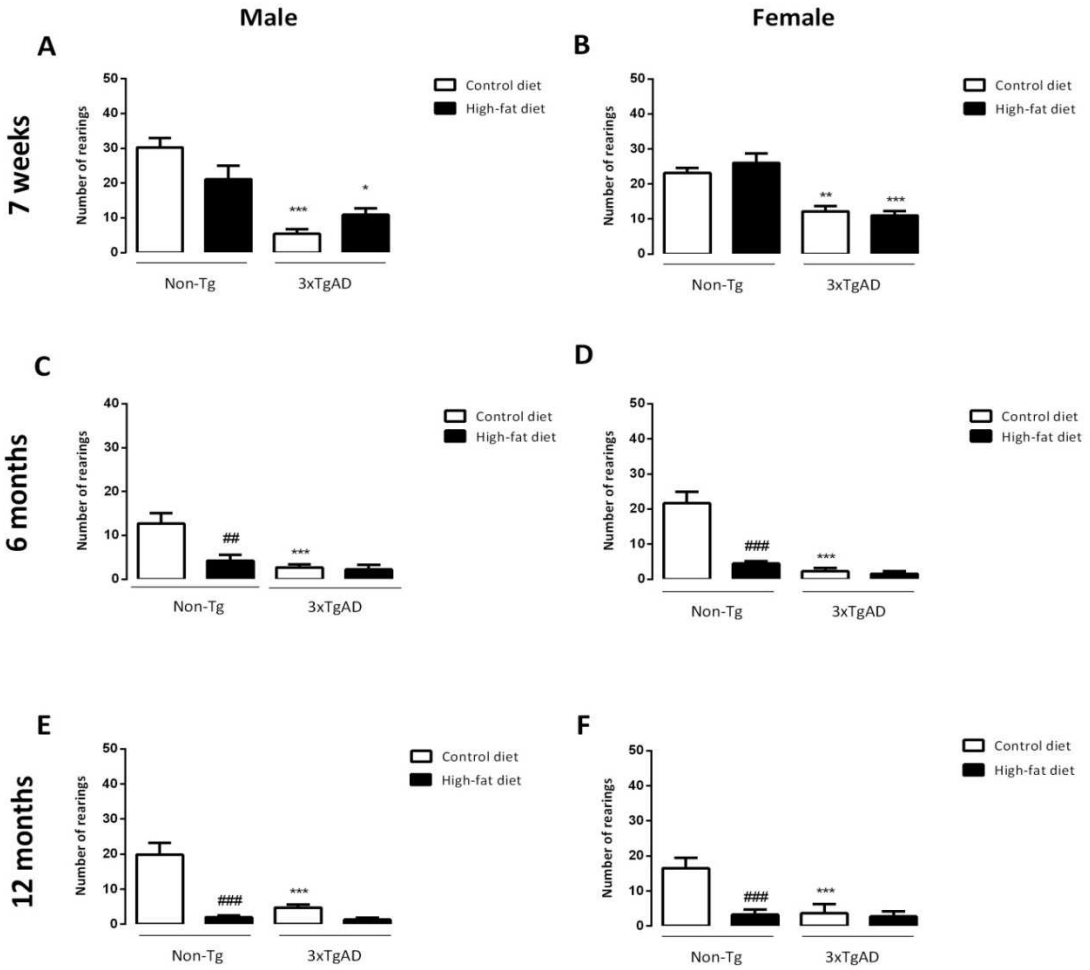
ICAM-1 measurement by ELISA

ICAM-1 levels were determined in hippocampal samples, diluted in 1 in 25, by an ICAM-1 mouse ELISA (DuoSet, R&D Systems, UK) according to the manufactures protocol.

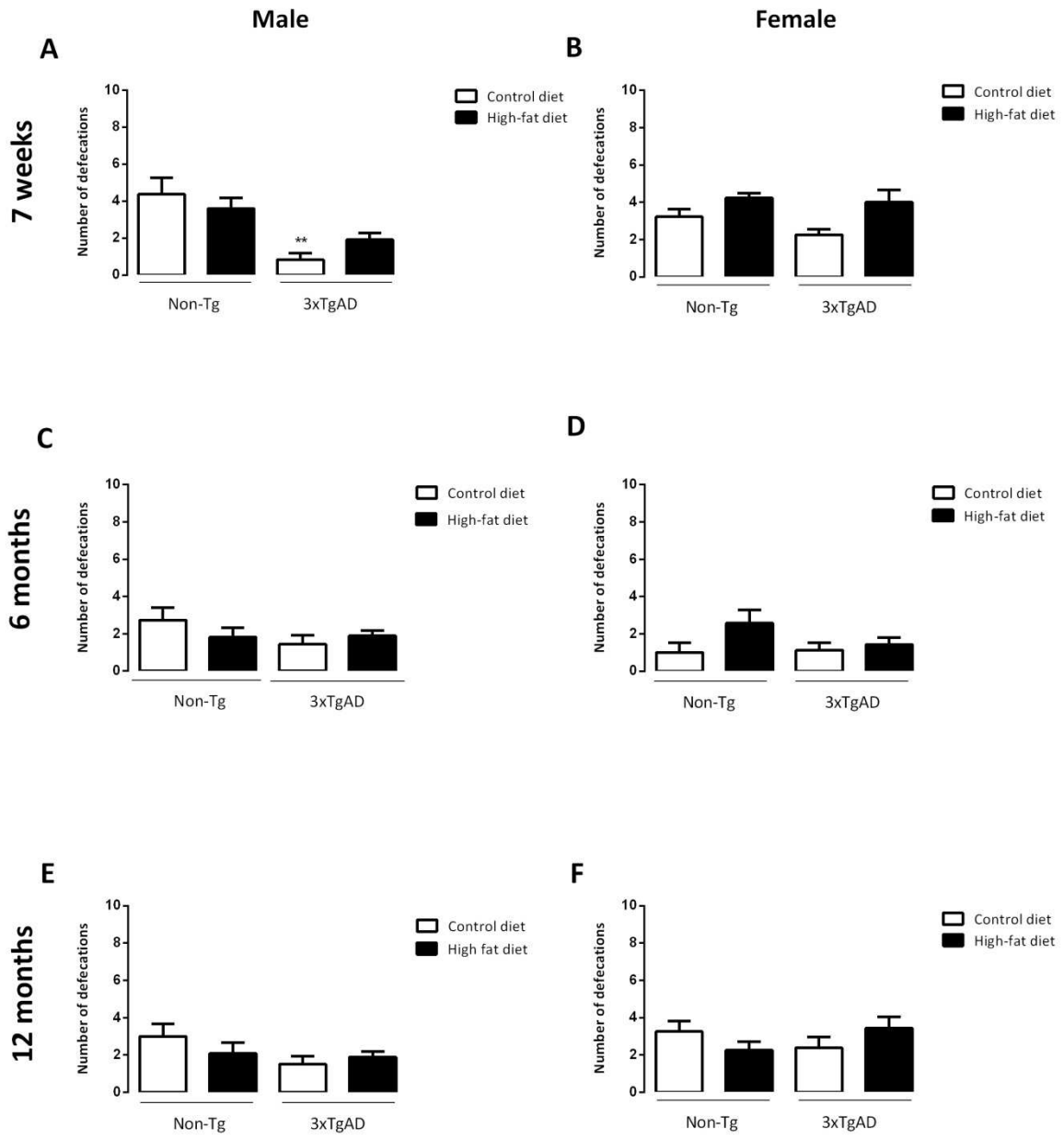
Western blotting

Sample buffer (12.5% 0.5 M Tris (pH 6.8), 12.5% glycerol, 2% sodium dodecyl sulfate (SDS), 5% β-mercaptoethanol, 0.25% bromophenol blue, 50% distilled water) was added to hippocampal samples (n=6-9) in a ratio of 1 to 5, and samples denatured at 95°C for 5 min. All proteins were resolved by SDS-PAGE on Tris-glycine acrylamide gels and transferred onto a nitrocellulose membrane (pore size 0.2 µm) at 15V for 45-60 min using a semi-dry transfer cell (Bio-Rad, UK). Following electrotransfer, the membranes were blocked for 1 h in phosphate buffered saline with tween 20 (PBST) and 5% (w/v) milk at room temperature (RT). Membranes were incubated with a rabbit primary antibody (PSD-95, synaptophysin or VDAC-1/porin, Abcam, UK) overnight at 4 °C, followed by horseradish peroxidase (HRP) conjugated secondary anti-rabbit antibody, both in PBST. Bound HRP conjugates were visualized with an enhanced chemiluminescence (ECL) reagent for 1 minute (GE Healthcare, UK), and the chemoluminescent signal produced (ImageQuant 350, GE Healthcare, UK). Densiometry quantification was performed by analysing the intensity of each band using ImageJ software (NIH, USA). Values were normalised to the house keeping protein β-actin (Sigma, UK).

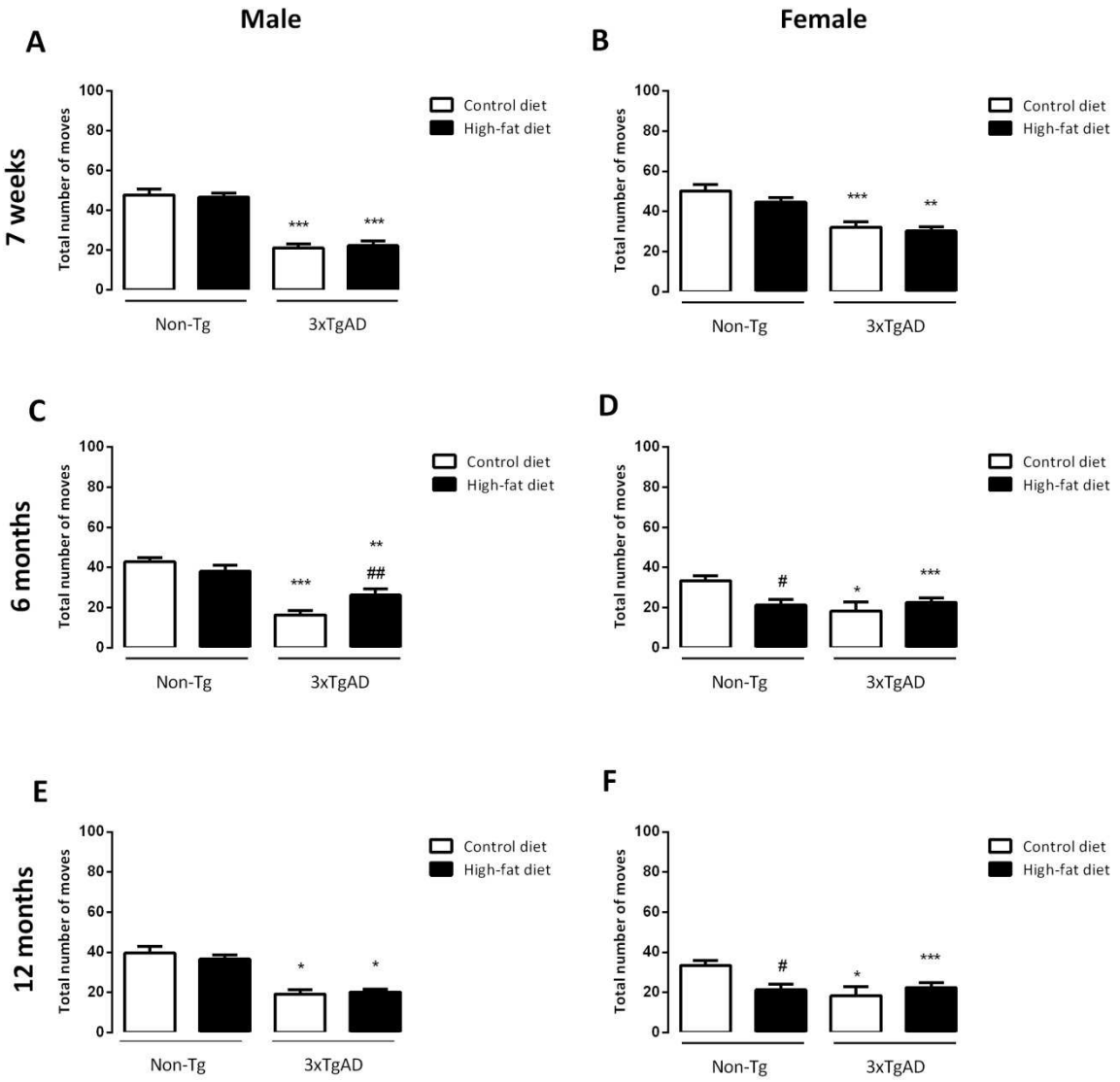
Supplementary Results



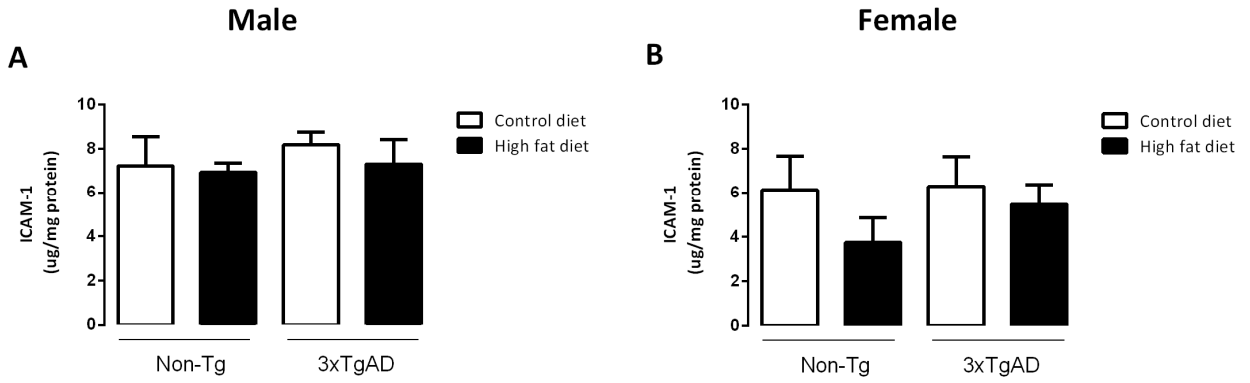
Supplementary Figure 4. High-fat diet leads to a reduction in the number of rearings in male and female Non-Tg in the open-field test after 6 months on diet. Groups of male and female Non-Tg control and 3xTgAD mice were maintained on a control or high-fat from 8 weeks of age. The number of rearings was assessed in the open-field test after 7 weeks and in a separate group of mice after 6 and 12 months on diet. Data are mean ± SEM, n = 9-12/group. ## $p < 0.01$, ### $p < 0.001$ versus control diet of the same genotype. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ versus Non-Tg on the same diet. Two-way ANOVA with Bonferroni's post hoc analysis.



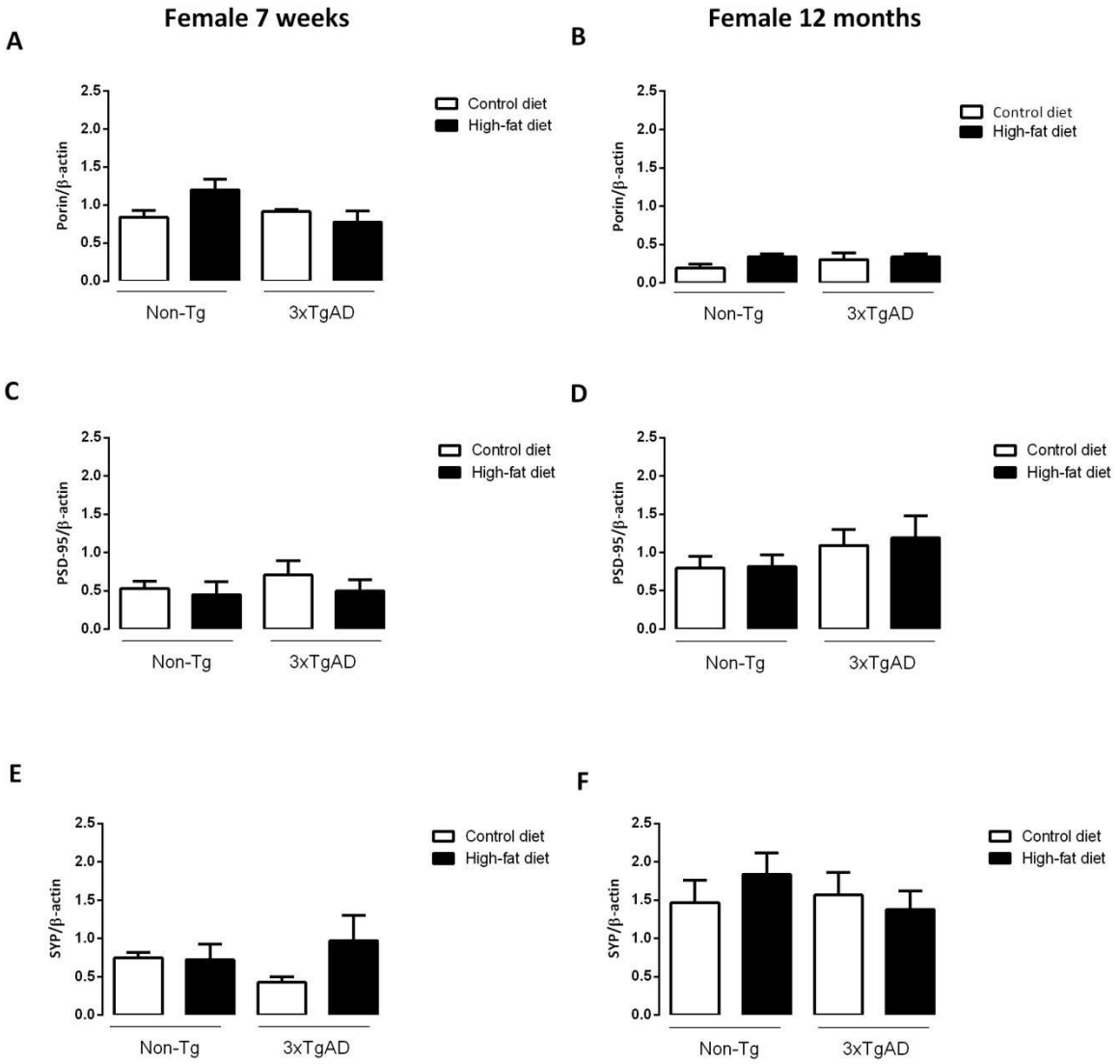
Supplementary Figure 5. Number of defecations in the open-field test in response to a high-fat diet in male and female Non-Tg control and 3xTgAD mice. Groups of male and female Non-Tg control and 3xTgAD mice were maintained on a control or high-fat from 8 weeks of age. The number of defecations was assessed in the open-field test after 7 weeks and in a separate group of mice after 6 and 12 months on diet. Data are mean ± SEM, n = 9-12/group. ** $p < 0.01$ versus Non-Tg on the same diet. Two-way ANOVA with Bonferroni's post hoc analysis.



Supplementary Figure 6. 3xTgAD mice move less and a high-fat diet reduces the number of moves in female Non-Tg mice in the Y-maze spontaneous alternation test. Groups of male and female Non-Tg control and 3xTgAD mice were maintained on a control or high-fat diet from 8 weeks of age..The total number of moves was assessed in the Y-maze spontaneous alternation test after 7 weeks, and in a separate group of mice after 6 and 12 months. Data are mean ± SEM, n = 9-12/group. # $p < 0.05$, ## $p < 0.01$ versus control diet of the same genoytype. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ versus Non-Tg on the same diet. Two-way ANOVA with Bonferroni's post hoc analysis



Supplementary Figure 7. A high-fat diet had no effect on hippocampal ICAM-1 levels in male and female Non-Tg and 3xTgAD mice. Groups of male and female Non-Tg control and 3xTgAD mice were maintained on a control or high-fat diet from 8 weeks of age. ICAM-1 protein levels were measured in hippocampal samples after 7 weeks on diet by ELISA. Data are mean ± SEM, n = 6-9/group.



Supplementary Figure 8. A high-fat diet did not affect synaptic proteins PSD-95 and synaptophysin or mitochondrial porin protein levels in hippocampus of female Non-Tg and 3xTgAD mice. Groups of female Non-Tg control and 3xTgAD mice were maintained on a control or high-fat diet from 8 weeks of age. Synaptic proteins, PSD-95 and synaptophysin, and mitochondrial protein porin were assessed by western blotting in hippocampal samples after 7 weeks and in a separate group of mice after 12 months on diet. Data Data expressed in arbitrary units are mean ± SEM, n = 6-9/group.

Chapter 4

Mitochondrial changes and synaptic loss after a high-fat diet and in a mouse model of Alzheimer's disease

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Mitochondrial changes and synaptic loss after a high-fat diet and in a mouse model of Alzheimer's disease

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Keywords: obesity; Alzheimer's disease, AD; control non-transgenic, Non-Tg; triple-transgenic mouse model of Alzheimer's disease, 3xTgAD; high-fat diet; reactive species of oxygen, ROS; mitochondria; electron transport chain, ETC; synapses; blood-brain barrier, BBB; electron microscopy, EM.

Abstract

Alzheimer's disease (AD) is the most common form of dementia that is associated with memory deficits and cognitive decline. One of the risk factors for developing AD later in life is obesity. Obesity, due to an increase consumption of diets rich in fatty acids, is also associated with memory impairment. In experimental animals a high-fat diet is known to cause memory deficits in control mice and in a mouse model of AD. However, the mechanisms underlying these effects of a high-fat diet on memory are unknown. Both AD and obesity can impair the blood brain barrier (BBB) and cause mitochondrial dysfunction. Mitochondria are known to be associated with neuronal function and are mainly present at sites of increased synaptic density. The aim of this study is to test the effect of a high-fat diet on the ultrastructure of the cerebrovasculature and neurones by studying the morphology of the BBB and mitochondria and the density of synapses. Male and female control non-transgenic (Non-Tg) and triple-transgenic AD (3xTgAD) mice were fed with a control or a high-fat diet for 6 months, starting at 8 weeks of age.

Ultrastructural analysis of the subiculum of the hippocampus showed no overall change in BBB structure in male or female Non-Tg and 3xTgAD mice. However, female 3xTgAD mice showed an increased percentage of elongated endothelial mitochondria, suggesting mitochondrial dysfunctional and stress. In hippocampal neuropil female 3xTgAD mice, but not male 3xTgAD mice, showed a reduced number of neuronal mitochondria with increased length and morphological abnormalities that were associated with reduced number of synapses. These same changes were observed in female Non-Tg mice fed with a high-fat diet, suggesting that female Non-Tg are more sensitive to changes induced by high-fat diet than males. Moreover, the effect of a high-fat diet was mainly seen in the hippocampus in both Non-Tg and 3xTgAD mice, but did not appear to exacerbate the changes seen in mitochondria or synapses in female 3xTgAD mice. Our data therefore demonstrate that the effects of a high-fat diet on mitochondria and synapses in female Non-Tg control mice are similar to changes observed in the 3xTgAD, suggesting that these changes could occur independently of amyloid (Aβ) dependent pathways but might play a role in the detrimental effect of a high-fat diet on cognition.

Introduction

Alzheimer's disease (AD) is the most common cause of dementia in the elderly and is characterised by a progressive and irreversible memory decline. Memory deficits are due to loss of synapses and neurones in brain regions involved in memory and learning processes such as the hippocampus, basal forebrain, entorhinal and temporal cortices [1, 2], though the hippocampus appears to be the main region affected [3]. The two major pathological hallmarks of AD are the presence of intracellular neurofibrillary tangles (NFT) and extracellular amyloid-β (Aβ) deposits. Such deposits, named senile plaques, contain a core of fibrillar Aβ and aggregate in extracellular spaces and in the cerebral vasculature [4]. In 1974, Miyakawa et al., demonstrated that the amyloid fibrils of the core of the senile plaques were mainly produced by the basement membrane of blood vessels [5, 6], demonstrating a relation between Aβ and the cerebrovasculature. Accumulation of Aβ in the cerebral microvasculature is known as cerebral amyloid angiopathy (CAA) [7] and is observed in more than 85% of AD cases [8]. Accumulation of fibrillar Aβ at the basement membrane of endothelial cells will consequently lead to degeneration of the same capillaries [9], causing endothelial dysfunction [10-13] and impairment of the blood brain barrier (BBB) [14-16].

The most known and accepted model for AD pathology is the amyloid cascade hypothesis [2, 4], however, another proposed hypothesis is the mitochondrial cascade hypothesis [17-19]. According to the latter, mitochondrial dysfunction occurs early in AD pathology preceding plaque formation and Aβ deposition [17].

Mitochondria play a fundamental role in the generation of energy, through production of adenosine triphosphate (ATP), but are also important in maintaining oxidative balance as they are responsible for producing radical species of oxygen (ROS) [20]. During AD progression there are reductions in ATP generation and enhanced ROS production in the mitochondria [20], which causes a vicious cycle, leading further deleterious effects in these organelles. In AD, this increase in ROS is caused by a disruption of the mitochondrial electron transport chain (ETC) [21]. Mitochondrial dysfunction has been described in both post-mortem AD brains [22-24] and in transgenic mouse models of the disease [25-27].

Mitochondrial-induced oxidative stress is also associated with alterations in mitochondria morphology, including dynamic processes of fusion and fission [28-30]. These dynamic processes allow the mitochondria to control their shape in order to adapt to several conditions, to exchange content between mitochondria regulating quality control and survival, to be transported to specific locations [31]. Therefore, mitochondria are able to adapt to changes in the cellular environment and perturbations in these dynamic processes have been demonstrated to contribute to neuronal dysfunction [31].

Mitochondria abundance is high in neurones, particularly at synapses, in order to supply the high energy demands of these cells [31]. As neurones are highly dependent on mitochondrial function, they are particularly sensitive to any disruption in mitochondrial dynamics [31]. Therefore, damaged mitochondria may be transported to synaptic terminals where they will produce low ATP levels and will lead to synaptic damage [32]. Mitochondria dysfunction has been linked to synaptic dysfunction [33, 34] and neurodegenerative diseases [31], and in the AD brain abnormalities in mitochondria structure are observed [35]. Loss of synapses and synaptic connectivity is also an early event of AD pathology [32, 36], and it is known that accumulation of Aβ in synapses causes synaptic damage and cognitive decline in AD [37-40], affecting in particular the hippocampus [36].

Several risk factors for AD have been proposed, including obesity, which is increasing worldwide and is usually due to increased consumption of high-fat diets [41-46]. Obesity, similar to AD, is also associated with endothelial dysfunction [47-52] and impairments in the BBB [53, 54]. It is known that obesity can trigger the onset of vascular disorders that affect BBB permeability later in life in humans [55] and that high-fat diets have an effect on BBB function in cognitively normal rodents by affecting tight junctions (TJs) integrity [53, 56, 57] and by suppressing the metabolic activity of BBB vessels through downregulation of several proteins [58]. In addition it is known that the effect of high-fat diets, in particular saturated fatty acids (SFA) on the BBB is related to Aβ transport and clearance. The BBB hosts the main Aβ transporters that permit the reciprocal transport of Aβ across the BBB [59, 60] and that SFA can induce and exacerbate cerebral amyloid deposition in both AD and control mice [61-64]. Several studies have also shown that BBB dysfunction is associated with impairment of hippocampal-dependent memory in rodents fed with a high-fat diet [51, 53, 65-68],

suggesting that BBB dysfunction could contribute to cognitive deficits associated with obesity and AD.

Diet-induced obesity studies have also been shown to affect memory and learning processes not only in control rodents [51, 69-76] but also in AD models [77-79], and it appears that SFA have a particular impact on hippocampal integrity [80]. Hippocampal neurones are known to have high metabolic demand, relying on oxidative phosphorylation and mitochondria for energy, which makes these neurones particular sensitive to damage if any of these energy sources are affected [80]. However, how high-fat diets can affect memory in healthy animals or make it worse in AD models is still unknown. Since mitochondria function is vital for healthy synapses and normal brain function, possible mechanisms that both AD and obesity share and that rely on mitochondria and vascular features, may be one of the reasons for memory impairments observed.

Therefore, the aim of this study was to assess the effects of a high-fat diet in control mice and an animal model of AD on 1) the morphology of the capillary endothelium; 2) endothelial and neuronal mitochondria number and morphology and 3) synaptic density, in order to understand the potential mechanism(s) involved in detrimental effects of a high-fat diet on memory. Male and female non-transgenic control mice (Non-Tg) and a triple transgenic mouse model of AD (3xTgAD) were maintained on a high-fat diet for 6 months and capillary endothelium, mitochondria and synaptic ultrastructure were evaluated by transmission electron microscopy (TEM) in the subiculum of the hippocampus and the cortex. In the hippocampus the subiculum is the principal target of the CA1 pyramidal cells and serves as the main output structure to the hippocampal formation [81]. The subiculum is the earliest and most affected region in AD patients [82-84]. In 3xTgAD mice, Aβ plaques are detected in the subiculum from 12-months of age but none are observed in the cortex at this time [77, 85-87]. Therefore we hypothesised that a high-fat diet will affect capillaries, mitochondria and synapses in the hippocampus and will have a less significant impact in the cortex of both Non-Tg and 3xTgAD mice.

Methods

Animals and diet

Male and female 3xTgAD, expressing mutant $PS1_{M146V}$, APP_{SWE} and Tau_{P301L}, and non-transgenic controls (Non-Tg), on a C57BL6 x 129SV background (originally created by Frank LaFerla (Irvine, CA, USA) [88]) were bred in-house and kept in standard housing conditions (humidity 50%-60%, temperature 21 ±1˚C, 12:12 hour light-dark) and given *ad libitum* access to food and water. After weaning Non-Tg and 3xTgAD male and female littermate mice were housed separately and kept on standard rodent chow (BK001, Special Diets Services, UK) until 8 weeks of age. Groups of mice were then randomly allocated to either a control diet (12% energy from fat, 5% fat content by weight, 0.78% saturated fatty acids, 58G7, Test Diets) or a high-fat diet (60% energy from fat, 35% fat content by weight, 13% saturated fatty acids, 58G9, Test Diets) for 6 months (n=3-4/group). Body weight was assessed at the end of diet. All animal experiments were carried out in accordance with the United Kingdom Animals (Scientific Procedures) Act 1986 and approved by the Home Office and the local Animal Ethical Review Group, University of Manchester.

Electron Microscopy

After 6 months on diet animals were terminally anesthetized with 3.5% isoflurane (30% O_2 and 70% N_2O) and prepared for TEM. A brief transcardiac perfusion with 0.9% saline was performed to remove circulating blood followed by perfusion with fixative containing 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1M phosphate buffer (pH 7.4). Brains were then dissected and post-fixed in fresh fixative for 24 h at 4°C. Coronal sections (100 µm thick) were cut on a Leica VT1000 vibrating-blade vibrotome and, in the coronal section of interest the subiculum of the hippocampus and primary somatosensory cortex, were microdissected. Samples were washed in cacodylate buffer containing 2 mM calcium chloride and post-fixed for 1 h with reduced osmium (O_2O_4 1% and $K_4Fe(CN)_6$ 1.5%), then incubated in 1% tannic acid in 0.1 M cacodylate buffer for 1 h and finally with 1% uranyl acetate in water overnight. Samples were dehydrated at room temperature with different grades of ethanol, from 20% to 100% for 5 min each, infiltrated with TAAB low viscosity resin (TAAB

Laboratories Equipment, UK) and polymerised for 24 h at 60°C. Ultrathin sections (70 nm) were then cut with Reichert Jung Ultracut E ultramicrotome (Reichert Technologies Life Sciences, USA), mounted on Athene 200 mesh thin bar copper grids (Agar Scientific, UK) and visualised with a FEI Technai 12 Biotwin Transmission Electron Microscope (FEI, USA) using a Gatan Orius SC1000 camera (Gatan, USA).

Data and statistical analyses

For each animal micrographs of 10 random capillaries, identified by their size and lack of smooth muscle, were taken for both the hippocampus and cortex and measurements made using ImageJ software (NIH, USA). The number of mitochondria in the capillary endothelium was counted and expressed as the average number of mitochondria per vessel. Mitochondrial length was determined by measuring the major length from one side to another. Healthy mitochondria were identified by their usual rounded shape with a dense matrix and the presence of cristae, and were variable in size ranging from 0.1 µm to 3 µm in length as previous described [89, 90]. In the present study, the average in length of control mitochondria was 0.5 µm, therefore mitochondria with 0.5 µm or above in length was classified as elongated mitochondria. The percentage of elongated mitochondria was calculated using the mitochondrial length measured. Other endothelial measurements were assessed in the hippocampus and included basal lamina thickness (nm), vessel lumen diameter (µm), vessel lumen roughness, % pericyte coverage, tight junction tortuosity and number of endothelial vesicles per µm of vessel wall as previously described using ImageJ [91, 92]. Briefly, basal lamina thickness was measured in 3 different locations and an average taken; vessel lumen diameter was calculated by taking into account the area of a circle (lumen) to extrapolate the diameter from its area; vessel lumen roughness was quantified by using the convex hull tool in ImageJ that estimates the actual perimeter of the lumen if the vessel was perfectly smooth; pericyte coverage was measured as the percentage of the lumen that was covered by a pericyte cell body or process; tight junction tortuosity was calculated by the ratio of the length of the tight junction from the luminal side to the basal lamina side, to the distance measured by drawing a straight line directly

from its start to its end, and the number of endothelial vesicles (caveolae) were counted and divided by the lumen perimeter in µm.

For neuronal mitochondria and synaptic density, 10 micrographs were taken for each animal of the neuropil of the hippocampus and cortex and measurements made using ImageJ. Here, the landmarks used to identify the correct field of view in each section consisted of avoiding nuclei and blood vessels, areas of myelination and increased dendritic density as previous described [93]. Using these guidelines the neuropil should be a synaptically dense region mostly composed by unmyelinated axons, dendrites, neurones and glial cells. In each field of view (63 μm^2), mitochondria number and length were determined as above, and the number of synapses was also quantified. Synapses were defined by the presence of synaptic vesicles and increased postsynaptic density due to their electron dense proprieties visible by TEM.

All micrographs were analysed blinded and in a random order. For each parameter, measurements from the 10 capillaries or fields of view (63 μm^2 for neurones) were averaged to give a mean for each animal and the group average obtained. Data were analysed using a two-way analysis of variance (ANOVA) followed by Bonferroni´s post-hoc test using SigmaPlot (Systat Software, Inc., San Jose, CA, USA). All data are expressed as the mean ± standard error of the mean (SEM). Statistical significance was taken when $p < 0.05$.

Results

High-fat diet increases body weight in both male and female Non-Tg mice but only in male 3xTgAD mice

After 6 months, a high-fat diet led to an increase in body weight in both male (255%) and female (150%) Non-Tg mice (Figure 1A, B). High-fat diet also led to an increase (188%) in body weight in male 3xTgAD mice (Figure 1A) but not in female 3xTgAD mice (Figure 1B).

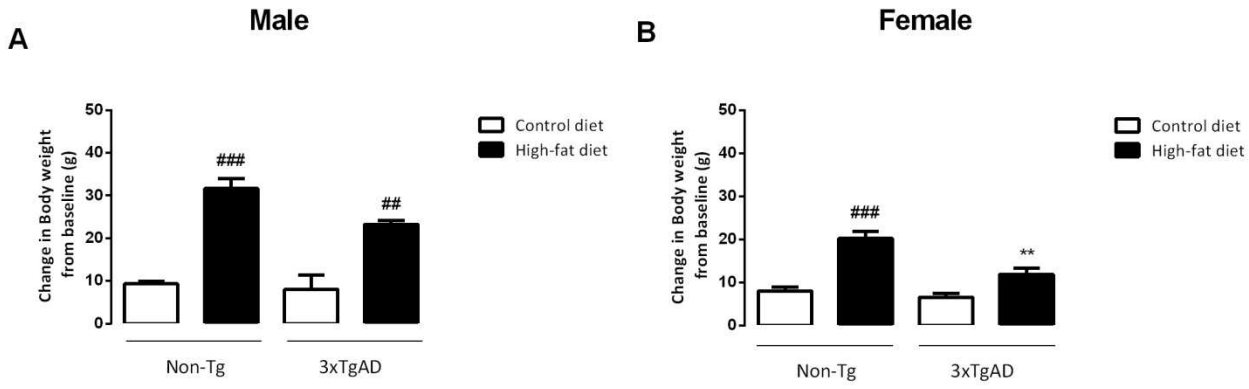

Figure 1. Effect of a high-fat diet on body weight of male and female Non-Tg and 3xTgAD mice. Male and female Non-Tg and 3xTgAD mice were maintained on a control or high-fat diet from 8 weeks of age and body weight was assessed after 6 months on diet. Data are mean ± SEM, n = 3-4/group. ### $p < 0.001$ versus control diet of the same genotype, ** $p < 0.01$ versus Non-Tg on the same diet. Two-way ANOVA with Bonferroni post hoc analysis.

High-fat diet had no impact on capillary structure in Non-Tg and 3xTgAD mice

No changes in any of the endothelial parameters measured were observed in response to a high-fat diet in male and female Non-Tg or 3xTgAD mice (Table 1). Also, no difference in the endothelial measures was seen between genotypes (Table 1). Due to the lack of effect of both diet and genotype in the hippocampus, one of the most affected areas in AD and in response to a high-fat diet, endothelial measurements in the capillary of the cortex were not assessed. In addition to the

quantitative assessment, no qualitative differences were observed in capillary structure in the hippocampus between Non-Tg and 3xTgAD mice on a control or a high-fat diet.

Table 1

High-fat diet does not affect the capillary structure in the hippocampus of male and female Non-Tg and 3xTgAD mice.

Male	Non-Tg		3xTgAD	
	Control	High-fat	Control	High-fat
Basal lamina thickness (nm)	83.5±3.8	81.4±8.3	84.1±7.9	82.5±6.2
Vessel lumen diameter (μm)	2.8±0.7	3.1±0.6	2.9±0.5	2.5±0.8
Vessel lumen roughness	1.2±0.06	1.1±0.03	1.1±0.06	1.2±0.06
Pericyte coverage (%)	21.9±3.0	23.2±2.1	24.5±1.6	19.3±1.9
Tight junction tortuosity	1.1±0.1	1.3±0.1	1.4±0.1	1.1±0.1
No. endothelial vesicles/μm of vessel wall	5±0	4±1	6±0	7±0

Female	Non-Tg		3xTgAD	
	Control	High-fat	Control	High-fat
Basal lamina thickness (nm)	70.6±7.3	69.4±1.4	69.6±6.9	69.7±2.4
Vessel lumen diameter (μm)	3.6±0.3	4.0±0.1	3.7±0.2	3.9±0.1
Vessel lumen roughness	1.04±0.02	1.02±0.01	1.11±0.09	1.03±0.01
Pericyte coverage (%)	21.2±1.3	24.5±1.2	23.0±5.4	24.4±2.0
Tight junction tortuosity	1.2±0.1	1.1±0.1	1.3±0.1	1.1±0.1
No. of endothelial vesicles/μm of vessel wall	3±0	3±0	3±0	3±0

Male and female Non-Tg control and 3xTgAD mice were kept on a control or high-fat diet from 8 weeks of age for 6 months. Capillary structure in the hippocampus was assessed in 10 random capillaries/animal. Data are mean ± SEM, n = 3-4/group.

High-fat diet increased the percentage of elongated endothelial mitochondria in the hippocampus of female Non-Tg and male 3xTgAD mice

The number of mitochondria in the capillary endothelium of the hippocampus and cortex of male and female Non-Tg and 3xTgAD mice remained unaltered by 6 months of high-fat diet. No difference in the number of mitochondria was also observed in control-fed male or female 3xTgAD mice when compared to Non-Tg mice on a control diet (Table 2). In control-fed female 3xTgAD mice, the percentage of elongated mitochondria was increased in the capillary endothelium of the hippocampus (Figure 2B, 2iii) and cortex (Figure 2D) when compared to control-fed Non-Tg mice. A high-fat diet also increased the number of elongated mitochondria in the endothelium of the hippocampus of female Non-Tg (Figure 2B, 2ii) and male 3xTgAD mice (Figure 2A). A high-fat diet had no effect on the percentage of elongated mitochondria in cortex of either Non-Tg or 3xTgAD mice of either sex (Figure 2C, D).

Table 2

High-fat diet does not alter mitochondria number in capillary endothelium of the hippocampus and cortex of male and female Non-Tg and 3xTgAD mice.

Hippocampus (mitochondria/vessel)	**Non-Tg**		**3xTgAD**	
	Control	High-fat	Control	High-fat
Male	3±1	4±1	5±1	3±0
Female	4±0	4±1	3±0	3±0
Cortex (mitochondria/vessel)	**Non-Tg**		**3xTgAD**	
	Control	High-fat	Control	High-fat
Male	2±0	2±0	4±1	3±0
Female	4±0	3±0	3±0	3±0

Male and female Non-Tg control and 3xTgAD mice were kept on a control or high-fat diet from 8 weeks of age for 6 months. Average number of endothelial mitochondria was assessed in 10 random capillaries/animal in the hippocampus and cortex. Data are mean ± SEM, n = 3-4/group.

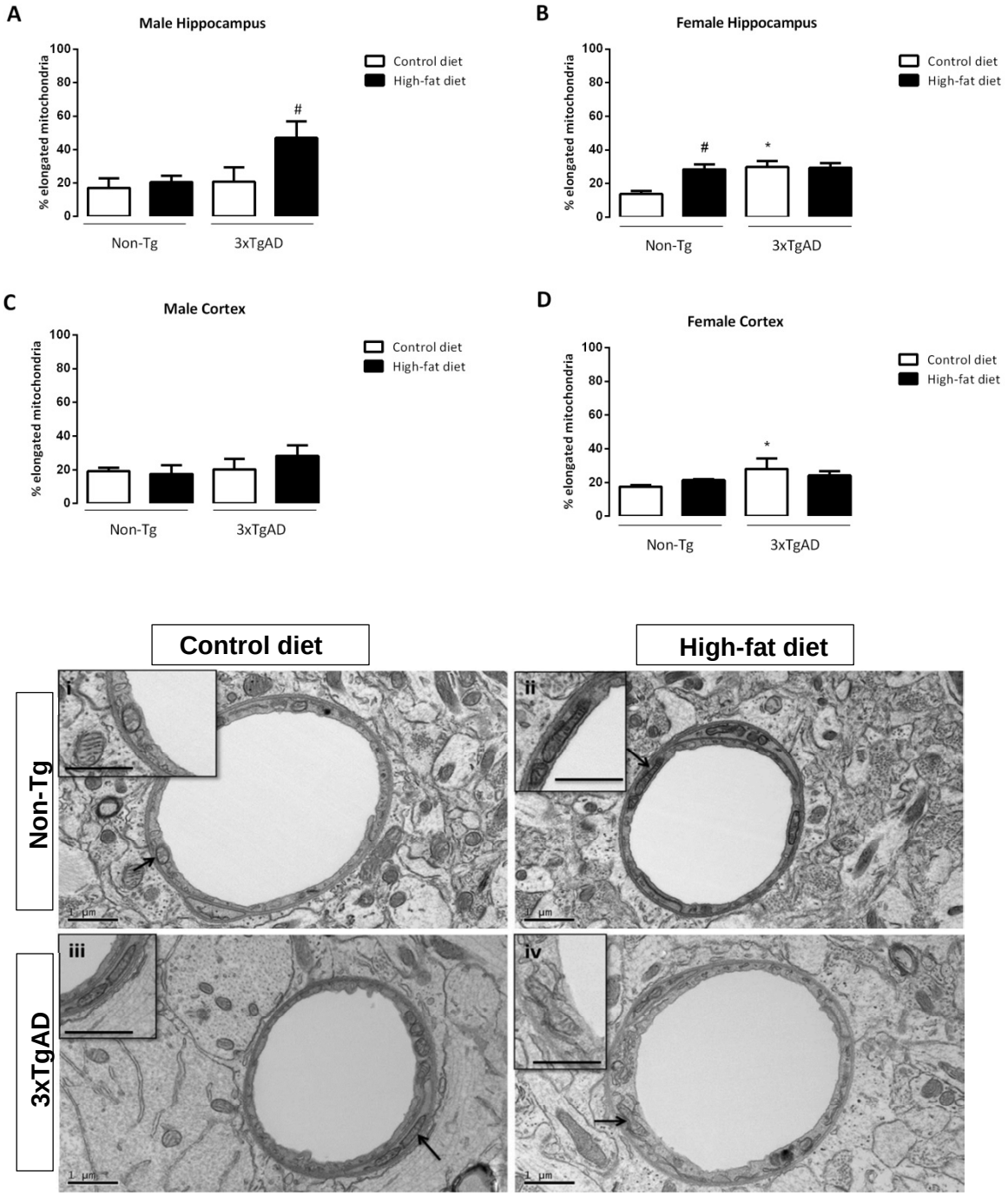

Figure 2. The length of endothelial mitochondria is increased by a high-fat diet in the hippocampus of female Non-Tg control mice and in control fed female 3xTgAD mice. Groups of male and female Non-Tg and 3xTgAD mice were maintained on a control or high-fat diet for 6 months and the length of endothelial mitochondria was measured and the percentage calculated in 10 random capillaries in the hippocampus and cortex **(A-D)**. Data are mean ± SEM, n = 3-4/group. # $p < 0.05$ versus control diet of the same genotype, * $p < 0.05$ versus Non-Tg on the same diet. Two-way ANOVA with Bonferroni post hoc analysis. (**i-iv**) Electron micrographs illustrating the presence of mitochondria (arrows) in the capillary endothelium in the

hippocampus of female Non-Tg and 3xTgAD mice fed a control or high-fat diet for 6 months. **(i)** Non-Tg mice fed on a control diet normal endothelial mitochondria with round shape and form; **(ii)** Non-Tg mice on a high-fat diet presented with longer mitochondria than control fed Non-Tg controls; **(iii)** 3xTgAD mice fed on a control diet with elongated endothelial mitochondria; **(iv)** High-fat fed 3xTgAD mice also presented with elongated mitochondria although not significant when calculated as percentage. Quantification is illustrated in the graphs above **(A-D)**. Scale bars: 1 µm, 220 nm (inset).

Neuronal mitochondrial number is reduced in female Non-Tg after a high-fat diet and in control-fed female 3xTgAD mice

In female Non-Tg mice fed a high-fat diet for 6 months a reduction (17%) in neuronal mitochondrial number in the hippocampus was detected compared to control fed mice (Table 3). A reduction in mitochondrial number (24%) was also observed in female 3xTgAD on a control diet versus Non-Tg control fed mice (Table 3). In female 3xTgAD mice, a high-fat diet led to an increase in mitochondrial number (36%) compared to control-fed 3xTgAD mice (Table 3). There was no effect of genotype or diet on neuronal mitochondria number in the hippocampus of male mice or the cortex of both male and female mice (Table 3).

Control fed female 3xTgAD mice had a greater percentage of elongated neuronal mitochondria in the hippocampus compared to Non-Tg mice on the same diet (Figure 3B, Figure 5C), but no difference was observed in the cortex (Figure 3D). There was no difference in mitochondrial length between male Non-Tg and 3xTgAD mice fed a control diet in the hippocampus or cortex (Figure 3A, C). After 6 months, a high-fat diet did not affect mitochondrial length in both Non-Tg and 3xTgAD male or female mice in either brain region (Figure 3A-D).

Table 3

High-fat diet reduces the number of neuronal mitochondria in the hippocampus of female Non-Tg control mice.

Hippocampus (mitochondria/63 µm²)	**Non-Tg**		**3xTgAD**	
	Control	High-fat	Control	High-fat
Male	21±1	20±3	19±3	17±3
Female	29±1	24±1[a]	22±1[i]	30±3[a]
Cortex (mitochondria/63 µm²)	**Non-Tg**		**3xTgAD**	
	Control	High-fat	Control	High-fat
Male	24±0	25±3	31±3	26±4
Female	25±2	23±1	24±2	26±2

Male and female Non-Tg control and 3xTgAD mice were kept on a control or high-fat diet from 8 weeks of age for 6 months. Average number of neuronal mitochondria was assessed in the hippocampus and cortex from 10 random fields of view (63 μm^2)/animal. Data are mean ± SEM, n = 3-4/group. [a] $p < 0.05$ versus control diet of the same genotype and sex, [i] $p < 0.05$ versus Non-Tg of the same sex and on the same diet. Two-way ANOVA with Bonferroni post hoc analysis.

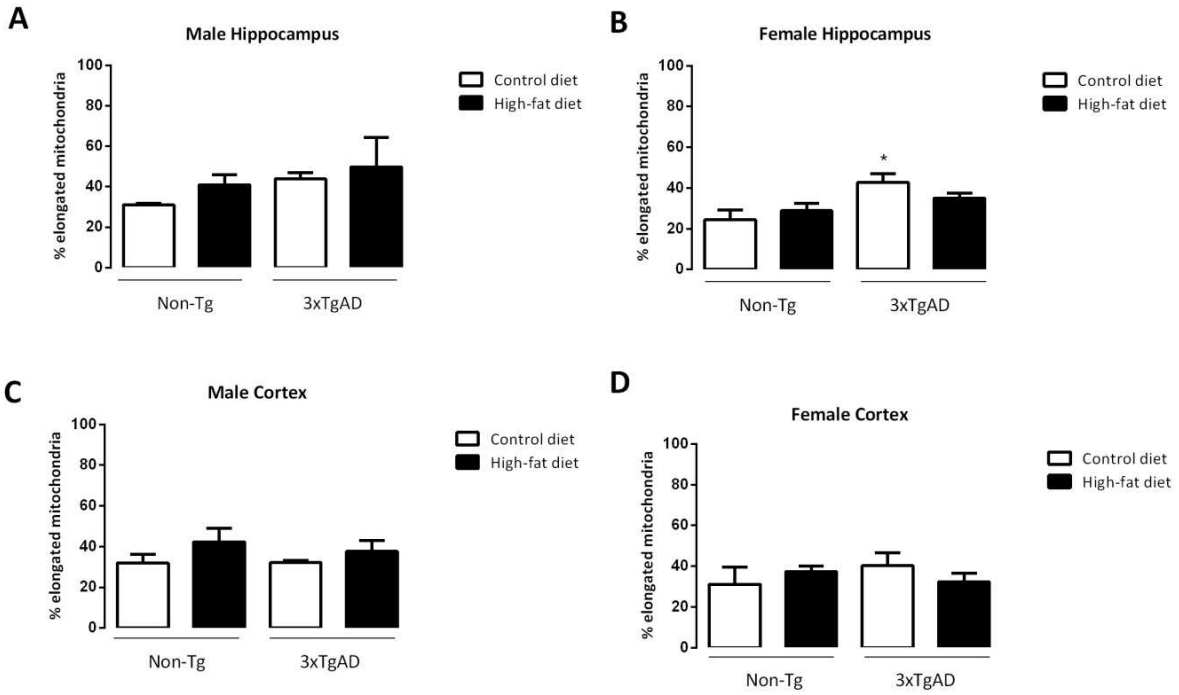

Figure 3. High-fat diet does not affect the length of neuronal mitochondria in the hippocampus or cortex of male and female Non-Tg and 3xTgAD mice. Groups of male and female Non-Tg and 3xTgAD mice were maintained on a control or high-fat diet for 6 months and the length of mitochondria was measured and the percentage of elongated neuronal mitochondria was calculated in the hippocampus and cortex in 10 different fields of view (63 μm^2)/animal. Data are mean ± SEM, n = 3-4/group. * $p < 0.05$ versus Non-Tg on the same diet. Two-way ANOVA with Bonferroni post hoc analysis.

Synaptic density is reduced in the hippocampus of female Non-Tg mice after a high-fat diet and in male and female control fed 3xTgAD mice

A high-fat diet led to a reduction in the number of synapses in the hippocampus of female Non-Tg mice (Figure 4B, Figure 5B) but had no effect in male Non-Tg mice or 3xTgAD mice of any sex. Fewer synapses were detected in the hippocampus of control-fed male and female 3xTgAD mice when compared to respective fed Non-Tg controls (Figure 4A, B and Figure 5). In the cortex, no difference in synaptic number was observed in any group assessed (Figure 4C, D).

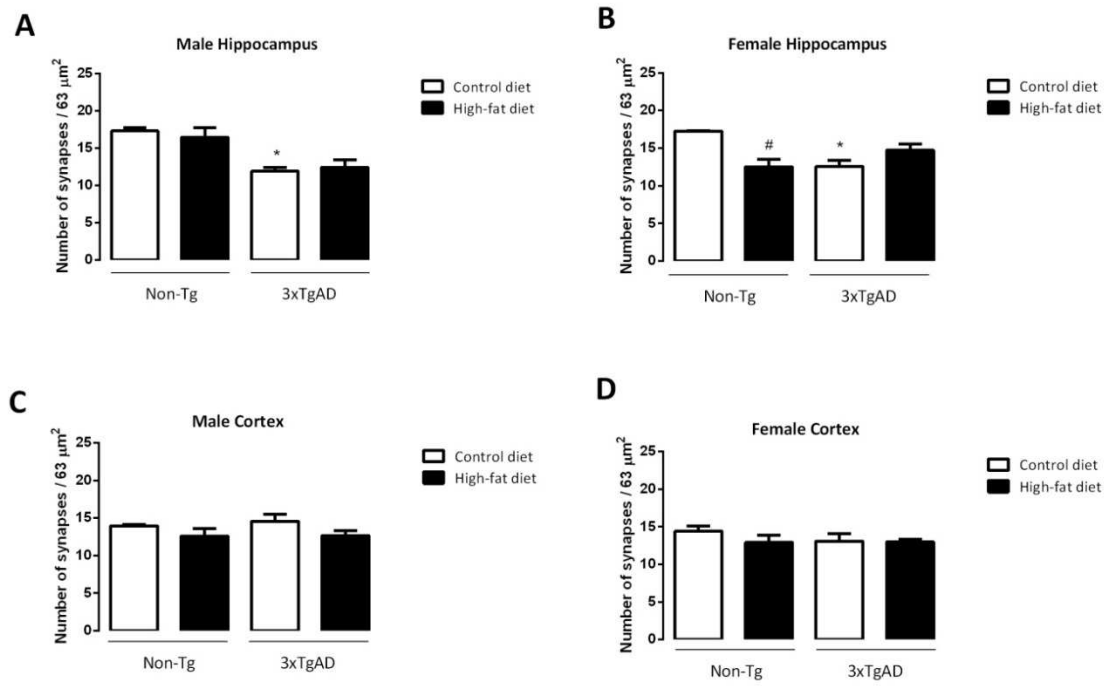

Figure 4. High-fat diet reduces synaptic number in the hippocampus of female Non-Tg control mice and a reduction in synaptic number is observed in control fed male and female 3xTgAD mice. Male and female Non-Tg control and 3xTgAD mice were kept on a control or high-fat diet from 8 weeks. After 6 months synaptic number was calculated in 10 different fields of view (63 μm^2)/animal in the hippocampus and cortex. Data are mean ± SEM, n = 3-4/group. # $p < 0.05$ versus control diet of the same genotype. * $p < 0.05$ versus Non-Tg on the same diet. Two-way ANOVA with Bonferroni post hoc analysis.

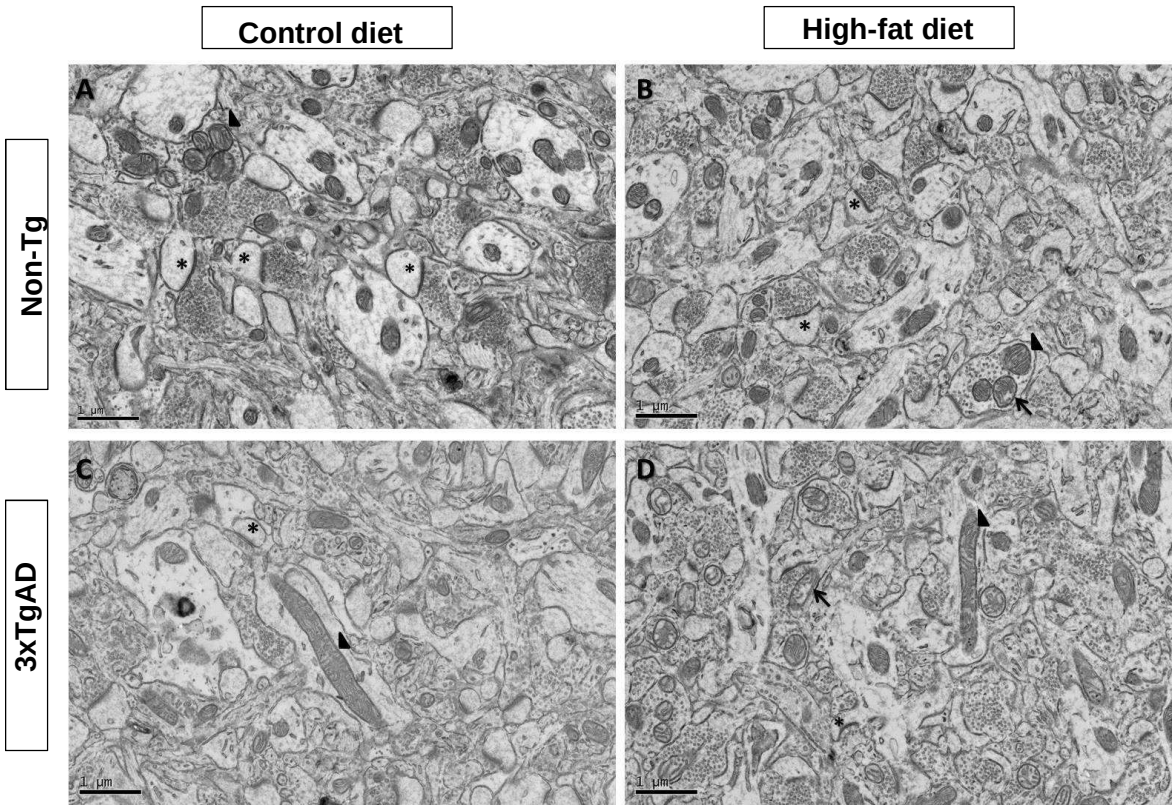

Figure 5. Synaptic number and neuronal mitochondria are affected by high-fat diet in the hippocampus of female Non-Tg, and in 3xTgAD mice fed with a control diet. (A) Synapses (asterisks) and neuronal mitochondria (arrow head) in the hippocampus of female Non-Tg mice on a control diet. **(B)** Non-Tg mice fed on a high-fat diet show decreased synaptic number (asterisks) and normal neuronal mitochondria (arrow head) with occasional abnormal mitochondria with holes and empty matrix (arrow). **(C)** 3xTgAD on a control diet show reduced number of synapses (asterisk) and elongated neuronal mitochondria (arrow head). **(D)** Synapses (asterisk) and abnormal mitochondria (arrow) from high-fat fed female 3xTgAD mice. Quantification of elongated mitochondria and synaptic number are illustrated in figure 3 and 4 respectively and neuronal mitochondrial number in Table 3. Scale bar: 1 µm.

3xTgAD mice have morphological abnormalities in neuronal mitochondrial in the hippocampus

Ultrastructural abnormalities in mitochondrial morphology were observed in both male and female 3xTgAD mice fed on a control or a high-fat diet. These mitochondrial structural abnormalities included elongation with swollen and disorganised cristae (Figure 6B), severe swollen cristae with less dense matrix (Figure 6C, E) and swollen mitochondria with severe loss of cristae and empty matrix (Figure 6D). None of these abnormal morphological changes were observed in Non-Tg mice fed either a control or high-fat diet.

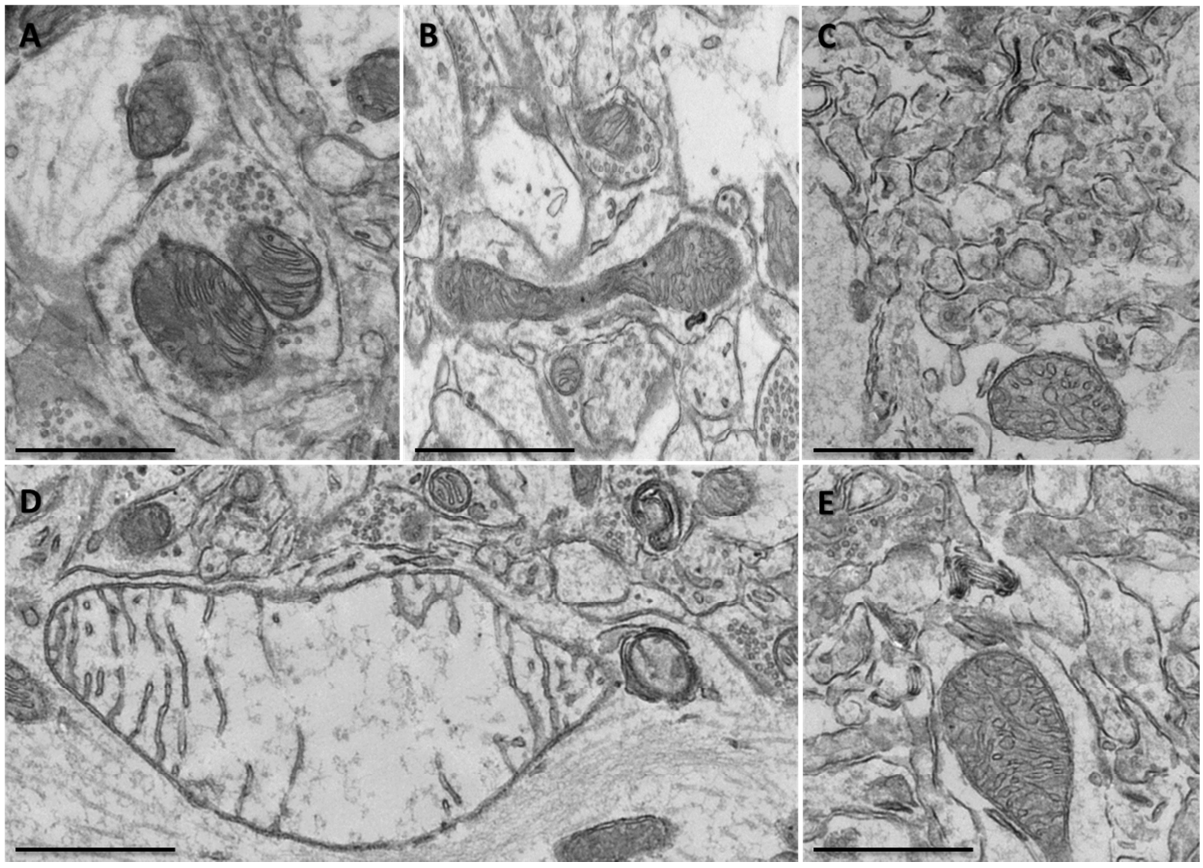

Figure 6. Neuronal mitochondrial abnormalities in the hippocampus of male and female 3xTgAD mice fed on a control or high-fat diet. In the hippocampus neuropil of Non-Tg mice normal mitochondria were observed to be round in shape containing packed cristae and dense matrix **(A)**. The following mitochondrial abnormalities were seen in 3xTgAD mice; elongated with swollen cristae **(B)**, distorted and swollen cristae and with less dense matrix **(C, E)** and swollen with severe loss of cristae with an empty matrix **(D)**. Scale bars: 1 µm.

High-fat diet led to morphological abnormalities in neuronal mitochondrial in female Non-Tg and 3xTgAD mice in the hippocampus

After 6 months a high-fat diet resulted in ultrastructural abnormalities in neuronal mitochondria in female Non-Tg (Figure 7A, B) and 3xTgAD (Figure 7C, D). In high-fat fed female mice, mitochondria had focal loss of cristae that was characterised by holes and empty spaces in the matrix. These neuronal mitochondrial structural changes were not observed in male high-fat fed Non-Tg or 3xTgAD mice.

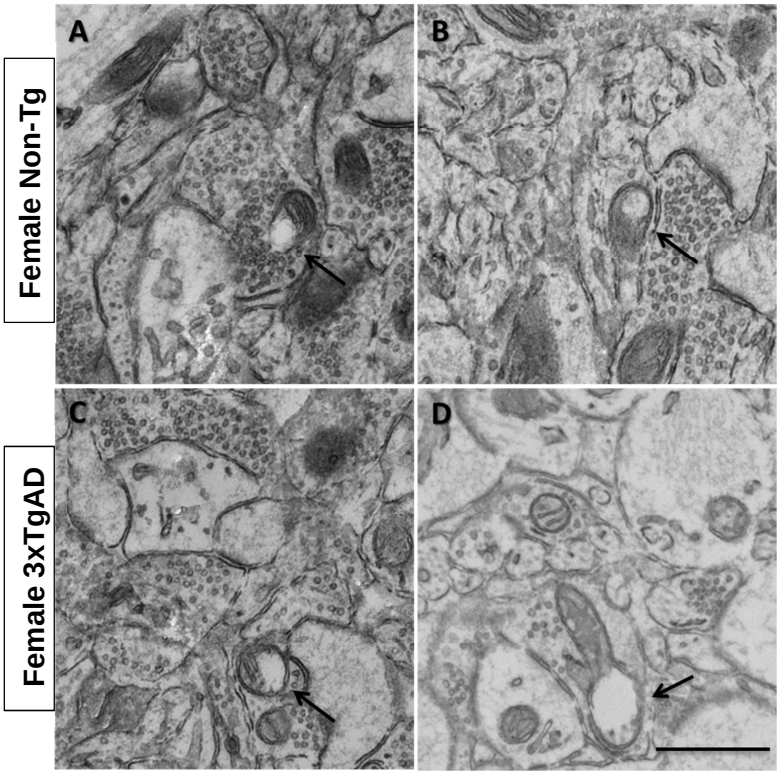

Figure 7. A high-fat diet affected neuronal mitochondrial morphology in the hippocampus of female Non-Tg and 3xTgAD mice. After 6 months a high-fat diet altered mitochondrial morphology in female Non-Tg **(A, B)** and 3xTgAD mice **(C, D)**. Mitochondria showed an absence of cristae, presented as holes and empty mitochondrial matrix (arrows). Scale bar: 500 nm.

Discussion

This study determined the effect of a high-fat diet on the ultrastructure of capillary endothelium, mitochondria and synapses in the hippocampus and cortex of both male and female Non-Tg and 3xTgAD mice. Our data demonstrated that a high-fat diet led to an increase of body weight in both male and female Non-Tg mice, but only in male 3xTgAD. The reason why female 3xTgAD do not gain weight with high-fat diet is unclear but previous studies have shown that body weight, metabolism and appetite in 3xTgAD mice is altered [94, 95].

High-fat diet led to mitochondrial changes in the capillary endothelium and neuropil, and a reduction in synaptic number in the hippocampus of female Non-Tg mice. These effects of a high-fat diet were similar to those observed in female 3xTgAD mice fed on a control diet. Changes in mitochondrial and synaptic morphology in response to a high-fat diet in Non-Tg mice were observed only in female mice and were specific to the hippocampus, as no differences were seen in male Non-Tg mice fed a high-fat diet in any brain region assessed.

A high-fat diet had no effect on the ultrastructure of the capillary endothelium in the hippocampus of either male or female Non-Tg and 3xTgAD mice. Although it is known that a high-fat diet and AD are associated with BBB impairment, such as increased BBB permeability, disruption of tight junction proteins, thickening of basement membrane and altered vessel structure in animal models [11, 14-16, 53, 54, 58, 68, 96, 97], our data showed no ultrastructural alterations in cerebral capillaries. However, changes in the morphology of the endothelial mitochondria were observed in female Non-Tg mice on a high-fat diet, without an overall change in mitochondrial number. Loss of endothelial mitochondria have been reported to be associated with BBB dysfunction in AD [97], since mitochondrial energy is required for active transport across the capillary [98], although we saw no reduction in mitochondrial number in 3xTgAD mice. Enlargement of the mitochondria may represent increased activity of these cells as a result of compensatory mechanisms against potential dysfunctional BBB [99], since increased density of enlarged mitochondria has been associated with BBB leakage [100]. This enlargement of mitochondria could be a protective way to maintain microvascular and neuronal integrity by trying to increase mitochondrial energy supply. Our results suggest that, although both AD and high-fat diet do not cause any structural changes in capillary

endothelium in the hippocampus, they have an impact on the number of elongated mitochondria. An increase in elongated mitochondria was observed in endothelium of the hippocampus of female Non-Tg and male 3xTgAD mice fed on a high-fat diet and in female control fed 3xTgAD mice in the hippocampus and cortex. It is known that in 11-month-old 3xTgAD mice the vascular volume is significant reduced in the hippocampus but not in cortex [101], with no difference in the functional integrity of the BBB [101, 102]. Such evidence could support our data showing no ultrastructural impairment of BBB integrity in the hippocampus of 3xTgAD mice. However, as we did not assess the levels of proteins involved in BBB integrity, such as TJs proteins, other BBB transporters, or BBB permeability, the effect of a high-fat diet on BBB function remains to be determined.

In contrast to the endothelium, the present study showed that the number of neuronal mitochondrial in the hippocampus was reduced in response to a high-fat diet in female Non-Tg mice and in female 3xTgAD mice fed on a control diet. The number of neuronal mitochondria has been reported to be reduced in diabetes [103], a common complication associated with both obesity [104] and AD [105-108]. Moreover, in female control fed 3xTgAD mice, this reduction in neuronal mitochondrial number was associated with an increase in the percentage of elongated mitochondria in the hippocampus, suggesting that in an AD mouse model not only the density but the morphology of mitochondria were affected, and that these effects are specific to the hippocampus.

Mitochondria can also undergo fission (division) and fusion, processes that can change mitochondrial morphology, number and shape [109]. Moreover, rates of mitochondrial fission and fusion are known to respond to changes in cellular metabolism [110]. Whilst fusion is stimulated by energy demands and stress, fission is normally activated to generate new organelles and facilitate quality control of mitochondria [110]. Tight regulation and control of such dynamic processes in mitochondria are essential for neuronal health, as an imbalance in these processes can lead to mitochondrial dysfunction and neuronal damage [111]. Some studies have demonstrated that in rodents, high-fat diets increase mitochondrial fusion in neurones [104], white adipose tissue [112] and hepatic cells [113]. Moreover, a recent study has shown that an elongated and tubular morphology, which is associated with mitochondrial fusion, can be a protective mechanism in mitochondria to avoid degradation by autophagy, in order to maintain mitochondrial numbers under

stress conditions [114]. In addition, an imbalance of mitochondrial dynamics in neurones, such as increase in fission and a decrease of fusion, have been shown to be early key factors in neurodegenerative disorders such as AD [105, 115-118]. Elongated mitochondria have also been observed in fibroblasts from AD patients and that this was likely due to a reduction in dynamin-related protein 1 (Drp1), a protein that regulates mitochondrial fission [119, 120]. However, in 3xTgAD mice mitochondrial fission in neurones is increased [116]. Therefore, our data supports that the balance of mitochondrial dynamics is affected by high-fat a diet and during AD progression, as the percentage of elongated mitochondria is altered. This suggests that changes in mitochondrial dynamics could possibly be due to an increase of fusion or a decrease of fission that are normally associated with an increase in mitochondrial length. However future research is needed to identify the particular pathway is affected.

Our results also indicate that changes in mitochondrial length and morphology observed in AD and after a high-fat diet are sex-dependent, affecting primarily female Non-Tg and 3xTgAD mice. Several studies have demonstrated that female rats have greater mitochondrial machinery (e.g. mitochondrial proteins) and therefore higher mitochondrial capacity and efficiency than male in several organs [121-123], including the brain [124, 125]. Moreover, it is also known that estrogen and progesterone can affect gene transcription of particular proteins involved in mitochondrial fusion and fission in a sex-specific way [126]. However, although we show changes in the number and morphology of mitochondria in female mice, we did not measure mitochondrial capacity and function.

In addition to a decrease in mitochondrial number in the brain of female high-fat fed Non-Tg control and 3xTgAD mice, our data also show that a high-fat diet can induce additional morphological changes in mitochondria of female Non-Tg and 3xTgAD mice, such as absence of cristae and empty mitochondrial matrix. Such results are consistent with findings from other experimental and animal studies where high-fat feeding [127-131], diabetes [132-134] and AD [135-137] led to swollen mitochondria with reduced electron dense matrix and loss of cristae integrity in neurones and other tissues.

In 3xTgAD control-fed mice other more severe morphological changes in mitochondria, such as swollen of the cristae and empty matrix, were observed in the hippocampus of both sexes. Several studies have demonstrated mitochondrial abnormalities in other AD mouse models [105, 136] but also in mitochondrial fractions from 3xTgAD mice [135]. Moreover, swollen mitochondria with swollen and irregular cristae or with loss or severe compromise of cristae integrity have been observed in the hippocampus of an animal model of prion disease, another neurodegenerative disorder [93]. Here we show for the first time that morphological changes in both male and female 3xTgAD are specific to the hippocampus, in particular to the subiculum, which is the region displaying the earliest severe pathology in AD patients [84, 138, 139]. However, we have previously shown extracellular Aβ plaques in the hippocampus of 3xTgAD mice are not detected until 12 months of age [77, 85-87, 94]. Mitochondrial changes therefore precede plaque formation in 3xTgAD mice in the subiculum and are associated with decrease in synaptic number. Moreover, since we observe also similar mitochondrial changes in high-fat fed Non-Tg mice it is unlikely that Aβ plaque formation is the cause of the ultrastructural changes in mitochondria seen here, and more likely that disruption of oxidative balance may play a role.

Synapses are sites of high energy demand associated with an abundance of mitochondria. Dysfunctional mitochondria would eventually be transported to synaptic terminals where they will produce low levels of ATP, causing detrimental effects on synapses and leading to synaptic degeneration [32-34]. Data in the present study showed that the number of synapses was reduced in both male and female 3xTgAD mice and that a high-fat diet caused a similar effect in female Non-Tg mice. Synaptic damage is known to occur in AD, and in AD patients there is a substantial decrease in the number of synapses [140, 141]. In addition, synaptic loss has also been shown in AD animal models [142-144] and *in vitro* in Aβ-treated neurones [40]. High-fat diets have also shown to effect synaptic integrity and decrease synaptic number in rodents [76, 96, 145], however here we show that a high-fat diet decreases synaptic density in female Non-Tg mice and that this effect is accompanied by a decrease in mitochondrial number in hippocampal neuropil. Moreover, mitochondria morphology is affected in hippocampal neuropil and a reduction in synaptic number is observed in 3xTgAD mice. Our findings therefore support that an imbalance in mitochondrial

dynamic processes, either as a cause or consequence of mitochondrial dysfunction, is associated with decrease synaptic number, as more damaged mitochondria will be transported to the synaptic site where they will produce more ROS and less ATP, causing synaptic degeneration.

In conclusion, our study demonstrated that the effects of a high-fat diet on mitochondria and synapses in female Non-Tg mice are similar to changes observed in the 3xTgAD mouse model of AD. These data therefore suggest that these changes in the brain are likely to occur independently of amyloid (Aβ) dependent pathways and might be responsible for the detrimental effect of a high-fat diet on cognition.

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References

1. Jacobsen, J.S., P. Reinhart, and M. Pangalos, *Current concepts in therapeutic strategies targeting cognitive decline and disease modification in Alzheimer's disease.* NeuroRX, 2005. **2**(4): p. 612-626.
2. Hardy, J. and D.J. Selkoe, *The Amyloid Hypothesis of Alzheimer's Disease: Progress and Problems on the Road to Therapeutics.* Science, 2002. **297**(5580): p. 353-356.
3. Marlatt, M.W. and P.J. Lucassen, *Neurogenesis and Alzheimer's disease: biology and pathophysiology in mice and men.* Curr Alzheimer Res 2010. **7**: p. 113–25.
4. Haass, C. and D.J. Selkoe, *Cellular processing of β-amyloid precursor protein and the genesis of amyloid β-peptide.* Cell, 1993. **75**(6): p. 1039-1042.
5. Miyakawa, T., et al., *Ultrastructure of capillary plaque-like degeneration in senile dementia.* Acta Neuropathologica, 1974. **29**(3): p. 229-236.
6. Miyakawa, T. and Y. Uehara, *Observations of amyloid angiopathy and senile plaques by the scanning electron microscope.* Acta Neuropathologica, 1979. **48**(2): p. 153-156.
7. Jellinger, K.A., *Alzheimer disease and cerebrovascular pathology: an update.* Journal of Neural Transmission, 2002. **109**(5-6): p. 813-836.
8. Attems, J., et al., *Review: Sporadic cerebral amyloid angiopathy.* Neuropathology and Applied Neurobiology, 2011. **37**(1): p. 75-93.
9. Miyakawa, T., *Vascular pathology in Alzheimer's disease.* Psychogeriatrics, 2010. **10**(1): p. 39-44.
10. Zlokovic, B.V., *Neurovascular pathways to neurodegeneration in Alzheimer's disease and other disorders.* Nat Rev Neurosci, 2011. **12**(12): p. 723-738.
11. Iadecola, C., et al., *SOD1 rescues cerebral endothelial dysfunction in mice overexpressing amyloid precursor protein.* Nat Neurosci, 1999. **2**(2): p. 157-161.
12. Niwa, K., G.A. Carlson, and C. Iadecola, *Exogenous Abeta1-40 Reproduces Cerebrovascular Alterations Resulting From Amyloid Precursor Protein Overexpression in Mice.* J Cereb Blood Flow Metab, 2000. **20**(12): p. 1659-1668.
13. Paul, J., S. Strickland, and J.P. Melchor, *Fibrin deposition accelerates neurovascular damage and neuroinflammation in mouse models of Alzheimer's disease.* The Journal of Experimental Medicine, 2007. **204**(8): p. 1999-2008.
14. Biron, K.E., et al., *Amyloid Triggers Extensive Cerebral Angiogenesis Causing Blood Brain Barrier Permeability and Hypervascularity in Alzheimer's Disease.* PLoS ONE, 2011. **6**(8): p. e23789.
15. Takeda, S., N. Sato, and R. Morishita, *Systemic inflammation, blood-brain barrier vulnerability and cognitive / non-cognitive symptoms in Alzheimer disease: Relevance to pathogenesis and therapy.* Frontiers in Aging Neuroscience, 2014. **6**.
16. Li, J.-c., et al., *Increased Permeability of the Blood-Brain Barrier and Alzheimer's Disease-Like Alterations in Slit-2 Transgenic Mice.* Journal of Alzheimer's Disease, 2015. **43**(2): p. 535-548.
17. Swerdlow, R.H., J.M. Burns, and S.M. Khan, *The Alzheimer's Disease Mitochondrial Cascade Hypothesis.* Journal of Alzheimer's Disease, 2010. **20**(0): p. 265-279.
18. Swerdlow, R.H., J.M. Burns, and S.M. Khan, *The Alzheimer's Disease Mitochondrial Cascade Hypothesis.* Journal of Alzheimer's disease : JAD, 2010. **20**(Suppl 2): p. 265-279.
19. Swerdlow, R.H. and S.M. Khan, *A "mitochondrial cascade hypothesis" for sporadic Alzheimer's disease.* Medical Hypotheses, 2004. **63**(1): p. 8-20.
20. Moreira, P.I., et al., *Mitochondrial dysfunction is a trigger of Alzheimer's disease pathophysiology.* Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease, 2010. **1802**(1): p. 2-10.
21. Wang, J., et al., *Increased oxidative damage in nuclear and mitochondrial DNA in Alzheimer's disease.* Journal of Neurochemistry, 2005. **93**(4): p. 953-962.
22. Devi, L., et al., *Accumulation of Amyloid Precursor Protein in the Mitochondrial Import Channels of Human Alzheimer's Disease Brain Is Associated with Mitochondrial Dysfunction.* The Journal of Neuroscience, 2006. **26**(35): p. 9057-9068.
23. Maurer, I., S. Zierz, and H.J. Möller, *A selective defect of cytochrome c oxidase is present in brain of Alzheimer disease patients.* Neurobiology of Aging, 2000. **21**(3): p. 455-462.

24. Hirai, K., et al., *Mitochondrial Abnormalities in Alzheimer's Disease.* The Journal of Neuroscience, 2001. **21**(9): p. 3017-3023.
25. Reddy, P.H., et al., *Gene expression profiles of transcripts in amyloid precursor protein transgenic mice: up-regulation of mitochondrial metabolism and apoptotic genes is an early cellular change in Alzheimer's disease.* Human Molecular Genetics, 2004. **13**(12): p. 1225-1240.
26. Caspersen, C., et al., *Mitochondrial Aβ: a potential focal point for neuronal metabolic dysfunction in Alzheimer's disease.* The FASEB Journal, 2005. **19**(14): p. 2040-2041.
27. Manczak, M., et al., *Mitochondria are a direct site of Aβ accumulation in Alzheimer's disease neurons: implications for free radical generation and oxidative damage in disease progression.* Human Molecular Genetics, 2006. **15**(9): p. 1437-1449.
28. Ozcan, C., et al., *Potassium channel openers protect cardiac mitochondria by attenuating oxidant stress at reoxygenation*. Vol. 282. 2002. H531-H539.
29. Santos, D., et al., *Diabetes and mitochondrial oxidative stress: A study using heart mitochondria from the diabetic Goto-Kakizaki rat.* Molecular and Cellular Biochemistry, 2003. **246**(1-2): p. 163-170.
30. Katakam, P.V.G., et al., *Myocardial preconditioning against ischemia-reperfusion injury is abolished in Zucker obese rats with insulin resistance*. Vol. 292. 2007. R920-R926.
31. Chen, H. and D.C. Chan, *Mitochondrial dynamics–fusion, fission, movement, and mitophagy–in neurodegenerative diseases.* Human Molecular Genetics, 2009. **18**(R2): p. R169-R176.
32. Reddy, P.H. and M.F. Beal, *Amyloid beta, mitochondrial dysfunction and synaptic damage: implications for cognitive decline in aging and Alzheimer's disease.* Trends in Molecular Medicine, 2008. **14**(2): p. 45-53.
33. Li, Z., et al., *The Importance of Dendritic Mitochondria in the Morphogenesis and Plasticity of Spines and Synapses.* Cell, 2004. **119**(6): p. 873-887.
34. Verstreken, P., et al., *Synaptic Mitochondria Are Critical for Mobilization of Reserve Pool Vesicles at Drosophila Neuromuscular Junctions.* Neuron, 2005. **47**(3): p. 365-378.
35. Baloyannis, S.J., *Mitochondrial alterations in Alzheimer's disease.* Journal of Alzheimer's Disease, 2006. **9**(2): p. 119-126.
36. Scheff, S.W. and D.A. Price, *Synaptic pathology in Alzheimer's disease: a review of ultrastructural studies.* Neurobiology of Aging, 2003. **24**(8): p. 1029-1046.
37. Gouras, G.K., et al., *Intraneuronal Aβ42 Accumulation in Human Brain.* The American Journal of Pathology, 2000. **156**(1): p. 15-20.
38. Tampellini, D., et al., *Synaptic Activity Reduces Intraneuronal Aβ, Promotes APP Transport to Synapses, and Protects against Aβ-Related Synaptic Alterations.* The Journal of Neuroscience, 2009. **29**(31): p. 9704-9713.
39. Bayer, T.A. and O. Wirths, *Intracellular accumulation of amyloid-beta - a predictor for synaptic dysfunction and neuron loss in Alzheimer's disease.* Frontiers in Aging Neuroscience, 2010. **2**.
40. Calkins, M.J. and P.H. Reddy, *Amyloid beta impairs mitochondrial anterograde transport and degenerates synapses in Alzheimer's disease neurons.* Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease, 2011. **1812**(4): p. 507-513.
41. Fitzpatrick, A.L., et al., *Midlife and late-life obesity and the risk of dementia: Cardiovascular health study.* Archives of Neurology, 2009. **66**(3): p. 336-342.
42. Hassing, L.B., et al., *Overweight in midlife and risk of dementia: a 40-year follow-up study.* Int J Obes, 2009. **33**(8): p. 893-898.
43. Whitmer, R.A., et al., *Obesity in middle age and future risk of dementia: a 27 year longitudinal population based study*. Vol. 330. 2005. 1360.
44. Whitmer, R.A., et al., *Central obesity and increased risk of dementia more than three decades later.* Neurology, 2008. **71**(14): p. 1057-64.
45. Beydoun, M.A., et al., *Association of Adiposity Status and Changes in Early to Mid-Adulthood With Incidence of Alzheimer's Disease.* American Journal of Epidemiology, 2008. **168**(10): p. 1179-1189.
46. Xu, W.L., et al., *Midlife overweight and obesity increase late-life dementia risk: a population-based twin study.* . Neurology, 2011. **76**: p.:1568–1574.

47. Steinberg, H.O., et al., *Obesity/insulin resistance is associated with endothelial dysfunction. Implications for the syndrome of insulin resistance.* The Journal of Clinical Investigation, 1996. **97**(11): p. 2601-2610.
48. Pierce, G.L., et al., *Nuclear Factor-κB Activation Contributes to Vascular Endothelial Dysfunction via Oxidative Stress in Overweight/Obese Middle-Aged and Older Humans.* Circulation, 2009. **119**(9): p. 1284-1292.
49. Campia, U., M. Tesauro, and C. Cardillo, *Human obesity and endothelium-dependent responsiveness.* British Journal of Pharmacology, 2012. **165**(3): p. 561-573.
50. Lynch, C.M., et al., *Nox2-Derived Superoxide Contributes to Cerebral Vascular Dysfunction in Diet-Induced Obesity.* Stroke, 2013. **44**(11): p. 3195-3201.
51. Tucsek, Z., et al., *Aging Exacerbates Obesity-induced Cerebromicrovascular Rarefaction, Neurovascular Uncoupling, and Cognitive Decline in Mice.* The Journals of Gerontology Series A: Biological Sciences and Medical Sciences, 2014.
52. Kitayama, J., et al., *Cerebral Vascular Dysfunction During Hypercholesterolemia.* Stroke, 2007. **38**(7): p. 2136-2141.
53. Kanoski, S.E., et al., *The Effects of a High-Energy Diet on Hippocampal Function and Blood-Brain Barrier Integrity in the Rat.* Journal of Alzheimer's Disease, 2010. **21**(1): p. 207-219.
54. Freeman, L.R. and A.-C.E. Granholm, *Vascular changes in rat hippocampus following a high saturated fat and cholesterol diet.* J Cereb Blood Flow Metab, 2012. **32**(4): p. 643-653.
55. Gustafson, D.R., et al., *Mid-life adiposity factors relate to blood–brain barrier integrity in late life.* Journal of Internal Medicine, 2007. **262**(6): p. 643-650.
56. Wolburg, H. and A. Lippoldt, *Tight junctions of the blood–brain barrier: development, composition and regulation.* Vascular Pharmacology, 2002. **38**(6): p. 323-337.
57. Takechi, R., et al., *Dietary fats, cerebrovasculature integrity and Alzheimer's disease risk.* Progress in Lipid Research, 2010. **49**(2): p. 159-170.
58. Ouyang, S., et al., *Diet-induced obesity suppresses expression of many proteins at the blood-brain barrier.* J Cereb Blood Flow Metab, 2014. **34**(1): p. 43-51.
59. Deane, R., Z. Wu, and B.V. Zlokovic, *RAGE (Yin) Versus LRP (Yang) Balance Regulates Alzheimer Amyloid β-Peptide Clearance Through Transport Across the Blood–Brain Barrier.* Stroke, 2004. **35**(11 suppl 1): p. 2628-2631.
60. Donahue, J., et al., *RAGE, LRP-1, and amyloid-beta protein in Alzheimer's disease.* Acta Neuropathologica, 2006. **112**(4): p. 405-415.
61. Oksman, M., et al., *Impact of different saturated fatty acid, polyunsaturated fatty acid and cholesterol containing diets on beta-amyloid accumulation in APP/PS1 transgenic mice.* Neurobiology of Disease, 2006. **23**(3): p. 563-572.
62. Refolo, L.M., et al., *Hypercholesterolemia Accelerates the Alzheimer's Amyloid Pathology in a Transgenic Mouse Model.* Neurobiology of Disease, 2000. **7**(4): p. 321-331.
63. Sparks, D.L., et al., *Induction of Alzheimer-like β-Amyloid Immunoreactivity in the Brains of Rabbits with Dietary Cholesterol.* Experimental neurology, 1994. **126**(1): p. 88-94.
64. Julien, C., et al., *High-fat diet aggravates amyloid-beta and tau pathologies in the 3xTg-AD mouse model.* Neurobiology of Aging, 2010. **31**(9): p. 1516-1531.
65. Molteni, R., et al., *A high-fat, refined sugar diet reduces hippocampal brain-derived neurotrophic factor, neuronal plasticity, and learning.* Neuroscience, 2002. **112**(4): p. 803-814.
66. Stranahan, A.M., et al., *Diet-induced insulin resistance impairs hippocampal synaptic plasticity and cognition in middle-aged rats.* Hippocampus, 2008. **18**(11): p. 1085-1088.
67. Kanoski, S.E. and T.L. Davidson, *Different patterns of memory impairments accompany short- and longer-term maintenance on a high-energy diet.* Journal of Experimental Psychology: Animal Behavior Processes, 2010. **36**(2): p. 313-319.
68. Davidson, T.L., et al., *The effects of a high-energy diet on hippocampal-dependent discrimination performance and blood–brain barrier integrity differ for diet-induced obese and diet-resistant rats.* Physiology & Behavior, 2012. **107**(1): p. 26-33.

69. Boitard, C., et al., *Impairment of hippocampal-dependent memory induced by juvenile high-fat diet intake is associated with enhanced hippocampal inflammation in rats.* Brain, Behavior, and Immunity, 2014. **40**(0): p. 9-17.
70. Valladolid-Acebes, I., et al., *Spatial memory impairment and changes in hippocampal morphology are triggered by high-fat diets in adolescent mice. Is there a role of leptin?* Neurobiology of Learning and Memory, 2013. **106**(0): p. 18-25.
71. Valladolid-Acebes, I., et al., *High-fat diets impair spatial learning in the radial-arm maze in mice.* Neurobiology of Learning and Memory, 2011. **95**(1): p. 80-85.
72. Heyward, F.D., et al., *Adult mice maintained on a high-fat diet exhibit object location memory deficits and reduced hippocampal SIRT1 gene expression.* Neurobiology of Learning and Memory, 2012. **98**(1): p. 25-32.
73. Tucker, K.R., et al., *Olfactory ability and object memory in three mouse models of varying body weight, metabolic hormones, and adiposity.* Physiology & Behavior, 2012. **107**(3): p. 424-432.
74. Reichelt, A.C., et al., *Dietary-induced obesity disrupts trace fear conditioning and decreases hippocampal reelin expression.* Brain, Behavior, and Immunity, 2015. **43**: p. 68-75.
75. Sobesky, J.L., et al., *High-fat diet consumption disrupts memory and primes elevations in hippocampal IL-1β, an effect that can be prevented with dietary reversal or IL-1 receptor antagonism.* Brain, Behavior, and Immunity, 2014. **42**: p. 22-32.
76. Arnold, S.E., et al., *High fat diet produces brain insulin resistance, synaptodendritic abnormalities and altered behavior in mice.* Neurobiology of Disease, 2014. **67**(0): p. 79-87.
77. 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.* Neurobiology of Aging, 2014. **35**(8): p. 1821-1832.
78. Barron, A.M., et al., *Sex-Specific Effects of High Fat Diet on Indices of Metabolic Syndrome in 3xTg-AD Mice: Implications for Alzheimer's Disease.* PLoS ONE, 2013. **8**(10): p. e78554.
79. Vandal, M., et al., *Insulin Reverses the High-Fat Diet–Induced Increase in Brain Aβ and Improves Memory in an Animal Model of Alzheimer Disease.* Diabetes, 2014.
80. Hsu, T.M. and S.E. Kanoski, *Blood-brain barrier disruption: mechanistic links between Western diet consumption and dementia.* Frontiers in Aging Neuroscience, 2014. **6**: p. 88.
81. Ding, S.-L., *Comparative anatomy of the prosubiculum, subiculum, presubiculum, postsubiculum, and parasubiculum in human, monkey, and rodent.* Journal of Comparative Neurology, 2013. **521**(18): p. 4145-4162.
82. Davies, C.A., et al., *A quantitative morphometric analysis of the neuronal and synaptic content of the frontal and temporal cortex in patients with Alzheimer's disease.* Journal of the Neurological Sciences, 1987. **78**(2): p. 151-164.
83. Apostolova, L.G., et al., *COnversion of mild cognitive impairment to alzheimer disease predicted by hippocampal atrophy maps.* Archives of Neurology, 2006. **63**(5): p. 693-699.
84. Falke, E., et al., *Subicular Dendritic Arborization in Alzheimer's Disease Correlates with Neurofibrillary Tangle Density.* The American Journal of Pathology, 2003. **163**(4): p. 1615-1621.
85. Perez, S.E., et al., *Cholinotrophic basal forebrain system alterations in 3xTg-AD transgenic mice.* Neurobiology of Disease, 2011. **41**(2): p. 338-352.
86. Cai, Y., et al., *BACE1 elevation is involved in amyloid plaque development in the triple transgenic model of Alzheimer's disease: Differential Aβ antibody labeling of early-onset axon terminal pathology.* Neurotoxicity research, 2012. **21**(2): p. 160-174.
87. Oddo, S., et al., *Blocking Aβ42 Accumulation Delays the Onset and Progression of Tau Pathology via the C Terminus of Heat Shock Protein70-Interacting Protein: A Mechanistic Link between Aβ and Tau Pathology.* The Journal of Neuroscience, 2008. **28**(47): p. 12163-12175.
88. Oddo, S., et al., *Triple-Transgenic Model of Alzheimer's Disease with Plaques and Tangles: Intracellular Aβ and Synaptic Dysfunction.* Neuron, 2003. **39**(3): p. 409-421.

89. Steenbergen, R., et al., *Disruption of the Phosphatidylserine Decarboxylase Gene in Mice Causes Embryonic Lethality and Mitochondrial Defects.* The Journal of biological chemistry, 2005. **280**(48): p. 40032-40040.
90. Rintoul, G.L., et al., *Glutamate Decreases Mitochondrial Size and Movement in Primary Forebrain Neurons.* The Journal of Neuroscience, 2003. **23**(21): p. 7881-7888.
91. Frank, R.N., S. Dutta, and M.A. Mancini, *Pericyte coverage is greater in the retinal than in the cerebral capillaries of the rat.* Investigative ophthalmology & visual science, 1987. **28**(7): p. 1086-91.
92. Jackman, K., et al., *Progranulin Deficiency Promotes Post-Ischemic Blood–Brain Barrier Disruption.* J Neurosci, 2013. **33**(50): p. 19579-19589.
93. Sisková, Z., et al., *Morphological and Functional Abnormalities in Mitochondria Associated with Synaptic Degeneration in Prion Disease.* The American Journal of Pathology, 2010. **177**(3): p. 1411-1421.
94. Knight, E.M., et al., *Hypermetabolism in a triple-transgenic mouse model of Alzheimer's disease.* Neurobiology of Aging, 2012. **33**(1): p. 187-193.
95. Adebakin, A., et al., *Impaired Satiation and Increased Feeding Behaviour in the Triple-Transgenic Alzheimer's Disease Mouse Model.* PLoS ONE, 2012. **7**(10): p. e45179.
96. Horvath, T.L., et al., *Synaptic input organization of the melanocortin system predicts diet-induced hypothalamic reactive gliosis and obesity.* Proceedings of the National Academy of Sciences, 2010. **107**(33): p. 14875-14880.
97. Kalaria, R.N., *Cerebral vessels in ageing and Alzheimer's disease.* Pharmacology & Therapeutics, 1996. **72**(3): p. 193-214.
98. Keuker, J.I.H., P.G.M. Luiten, and E. Fuchs, *Capillary changes in hippocampal CA1 and CA3 areas of the aging rhesus monkey.* Acta Neuropathol., 2000. **100**: p. 665-672.
99. Farkas, E. and P.G.M. Luiten, *Cerebral microvascular pathology in aging and Alzheimer's disease.* Progress in Neurobiology, 2001. **64**(6): p. 575-611.
100. Chen, B., et al., *Severe Blood–Brain Barrier Disruption and Surrounding Tissue Injury.* Stroke, 2009. **40**(12): p. e666-e674.
101. Bourasset, F., et al., *Reduction of the cerebrovascular volume in a transgenic mouse model of Alzheimer's disease.* Neuropharmacology, 2009. **56**(4): p. 808-813.
102. Do, T.M., et al., *Altered cerebral vascular volumes and solute transport at the blood–brain barriers of two transgenic mouse models of Alzheimer's disease.* Neuropharmacology, 2014. **81**(0): p. 311-317.
103. Jing, Y.H., et al., *Neurodegeneration in Streptozotocin-Induced Diabetic Rats Is Attenuated by Treatment with Resveratrol.* Neuroendocrinology, 2013. **98**(2): p. 116-127.
104. Dietrich, Marcelo O., Z.-W. Liu, and Tamas L. Horvath, *Mitochondrial Dynamics Controlled by Mitofusins Regulate Agrp Neuronal Activity and Diet-Induced Obesity.* Cell, 2013. **155**(1): p. 188-199.
105. Calkins, M.J., et al., *Impaired mitochondrial biogenesis, defective axonal transport of mitochondria, abnormal mitochondrial dynamics and synaptic degeneration in a mouse model of Alzheimer's disease.* Human Molecular Genetics, 2011. **20**(23): p. 4515-4529.
106. Fattoretti, P., et al., *Decreased Numeric Density of Succinic Dehydrogenase-Positive Mitochondria in CA1 Pyramidal Neurons of 3xTg-AD Mice.* Rejuvenation Research, 2009. **13**(2-3): p. 144-147.
107. Wang, X., et al., *Amyloid-β overproduction causes abnormal mitochondrial dynamics via differential modulation of mitochondrial fission/fusion proteins.* Proceedings of the National Academy of Sciences, 2008. **105**(49): p. 19318-19323.
108. Godoy, J.A., et al., *SIRT1 Protects Dendrites, Mitochondria and Synapses from Aβ Oligomers in Hippocampal Neurons.* J Alzheimers Dis Parkinsonism, 2013. **3**(1-9).
109. Cerveny, K.L., et al., *Regulation of mitochondrial fusion and division.* Trends in Cell Biology, 2007. **17**(11): p. 563-569.
110. Youle, R.J. and A.M. van der Bliek, *Mitochondrial Fission, Fusion, and Stress.* Science, 2012. **337**(6098): p. 1062-1065.
111. Reddy, P.H., et al., *Dynamin-related protein 1 and mitochondrial fragmentation in neurodegenerative diseases.* Brain Research Reviews, 2011. **67**(1–2): p. 103-118.

112. Amengual-Cladera, E., et al., *High-fat diet feeding induces a depot-dependent response on the pro-inflammatory state and mitochondrial function of gonadal white adipose tissue.* British Journal of Nutrition, 2013. **109**(03): p. 413-424.
113. Lionetti, L., et al., *High-Lard and High-Fish-Oil Diets Differ in Their Effects on Function and Dynamic Behaviour of Rat Hepatic Mitochondria.* PLoS ONE, 2014. **9**(3): p. e92753.
114. Rambold, A.S., et al., *Tubular network formation protects mitochondria from autophagosomal degradation during nutrient starvation.* Proceedings of the National Academy of Sciences, 2011. **108**(25): p. 10190-10195.
115. Itoh, K., et al., *Mitochondrial dynamics in neurodegeneration.* Trends in Cell Biology, 2013. **23**(2): p. 64-71.
116. Manczak, M. and P.H. Reddy, *Abnormal interaction between the mitochondrial fission protein Drp1 and hyperphosphorylated tau in Alzheimer's disease neurons: implications for mitochondrial dysfunction and neuronal damage.* Human Molecular Genetics, 2012. **21**(11): p. 2538-2547.
117. Manczak, M., M.J. Calkins, and P.H. Reddy, *Impaired mitochondrial dynamics and abnormal interaction of amyloid beta with mitochondrial protein Drp1 in neurons from patients with Alzheimer's disease: implications for neuronal damage.* Human Molecular Genetics, 2011. **20**(13): p. 2495-2509.
118. Cho, D.-H., T. Nakamura, and S. Lipton, *Mitochondrial dynamics in cell death and neurodegeneration.* Cellular and Molecular Life Sciences, 2010. **67**(20): p. 3435-3447.
119. Wang, X., et al., *Dynamin-Like Protein 1 Reduction Underlies Mitochondrial Morphology and Distribution Abnormalities in Fibroblasts from Sporadic Alzheimer's Disease Patients.* The American Journal of Pathology, 2008. **173**(2): p. 470-482.
120. Wang, X., et al., *Impaired Balance of Mitochondrial Fission and Fusion in Alzheimer's Disease.* The Journal of Neuroscience, 2009. **29**(28): p. 9090-9103.
121. Justo, R., et al., *Gender dimorphism in rat liver mitochondrial oxidative metabolism and biogenesis*. Vol. 289. 2005. C372-C378.
122. Valle, A., et al., *Sexual dimorphism in liver mitochondrial oxidative capacity is conserved under caloric restriction conditions*. Vol. 293. 2007. C1302-C1308.
123. Rodríguez-Cuenca, S., et al., *Sex-dependent Thermogenesis, Differences in Mitochondrial Morphology and Function, and Adrenergic Response in Brown Adipose Tissue.* Journal of Biological Chemistry, 2002. **277**(45): p. 42958-42963.
124. Guevara, R., et al., *Sex-dependent differences in aged rat brain mitochondrial function and oxidative stress.* Free Radical Biology and Medicine, 2009. **46**(2): p. 169-175.
125. Guevara, R., et al., *Age and Sex-Related Changes in Rat Brain Mitochondrial Function.* Cell Physiol Biochem, 2011. **27**: p. 201-206.
126. Arnold, S., G.W. de Araújo, and C. Beyer, *Gender-specific regulation of mitochondrial fusion and fission gene transcription and viability of cortical astrocytes by steroid hormones.* Journal of Molecular Endocrinology, 2008. **41**(5): p. 289-300.
127. Pipatpiboon, N., et al., *PPARγ Agonist Improves Neuronal Insulin Receptor Function in Hippocampus and Brain Mitochondria Function in Rats with Insulin Resistance Induced by Long Term High-Fat Diets.* Endocrinology, 2012. **153**(1): p. 329-338.
128. Bonnard, C., et al., *Mitochondrial dysfunction results from oxidative stress in the skeletal muscle of diet-induced insulin-resistant mice.* The Journal of Clinical Investigation, 2008. **118**(2): p. 789-800.
129. Du, Z., et al., *A long-term high-fat diet increases oxidative stress, mitochondrial damage and apoptosis in the inner ear of d-galactose-induced aging rats.* Hearing Research, 2012. **287**(1–2): p. 15-24.
130. Kiki, I., et al., *Effect of High Fat Diet on the Volume of Liver and Quantitative Feature of Kupffer Cells in the Female Rat: A Stereological and Ultrastructural Study.* Obesity Surgery, 2007. **17**(10): p. 1381-1388.

131. Reddy, S.S., et al., *Altered ubiquitin-proteasome system leads to neuronal cell death in a spontaneous obese rat model.* Biochimica et Biophysica Acta (BBA) - General Subjects, 2014. **1840**(9): p. 2924-2934.
132. Hernández-Fonseca, J.P., et al., *Structural and Ultrastructural Analysis of Cerebral Cortex, Cerebellum, and Hypothalamus from Diabetic Rats.* Experimental Diabetes Research, 2009. **2009**: p. 12.
133. Ye, L., F. Wang, and R.-H. Yang, *Diabetes impairs learning performance and affects the mitochondrial function of hippocampal pyramidal neurons.* Brain Research, 2011. **1411**(0): p. 57-64.
134. Zhou, J., et al., *Expression changes of growth-associated protein-43 (GAP-43) and mitogen-activated protein kinase phosphatase-1 (MKP-1) and in hippocampus of streptozotocin-induced diabetic cognitive impairment rats.* Experimental neurology, 2007. **206**(2): p. 201-208.
135. Carvalho, C., et al., *Metabolic Alterations Induced by Sucrose Intake and Alzheimer's Disease Promote Similar Brain Mitochondrial Abnormalities.* Diabetes, 2012. **61**(5): p. 1234-1242.
136. Trushina, E., et al., *Defects in Mitochondrial Dynamics and Metabolomic Signatures of Evolving Energetic Stress in Mouse Models of Familial Alzheimer's Disease.* PLoS ONE, 2012. **7**(2): p. e32737.
137. Zou, Y., et al., *Hypoxia-Up-Regulated Mitochondrial Movement Regulator Does Not Contribute to the APP/PS1 Double Transgenic Mouse Model of Alzheimer's Disease.* Dement Geriatr Cogn Disord, 2013. **36**: p. 137-145.
138. Davies, D.C., et al., *The effect of age and Alzheimer's disease on pyramidal neuron density in the individual fields of the hippocampal formation.* Acta Neuropathologica, 1992. **83**(5): p. 510-517.
139. Šimić, G., et al., *Volume and number of neurons of the human hippocampal formation in normal aging and Alzheimer's disease.* The Journal of Comparative Neurology, 1997. **379**(4): p. 482-494.
140. Scheff, S.W., et al., *Synaptic loss in the inferior temporal gyrus in mild cognitive impairment and Alzheimer's disease.* J Alzheimers Dis, 2011. **24**: p. 547–557.
141. Selkoe, D.J., *Alzheimer's Disease Is a Synaptic Failure.* Science, 2002. **298**(5594): p. 789-791.
142. Alonso-Nanclares, L., et al., *Synaptic Changes in the Dentate Gyrus of APP/PS1 Transgenic Mice Revealed by Electron Microscopy.* Journal of Neuropathology & Experimental Neurology, 2013. **72**(5): p. 386-395 10.1097/NEN.0b013e31828d41ec.
143. Esfandiary, E., et al., *Novel effects of Rosa damascena extract on memory and neurogenesis in a rat model of Alzheimer's disease.* Journal of Neuroscience Research, 2014. **92**(4): p. 517-530.
144. Zhang, Z., et al., *7,8-Dihydroxyflavone Prevents Synaptic Loss and Memory Deficits in a Mouse Model of Alzheimer/'s Disease.* Neuropsychopharmacology, 2014. **39**(3): p. 638-650.
145. Calvo-Ochoa, E., et al., *Short-term high-fat-and-fructose feeding produces insulin signaling alterations accompanied by neurite and synaptic reduction and astroglial activation in the rat hippocampus.* J Cereb Blood Flow Metab, 2014. **34**(6): p. 1001-1008.

Supplemental Material

Mitochondrial changes and synaptic loss after a high-fat diet and in a mouse model of Alzheimer's disease

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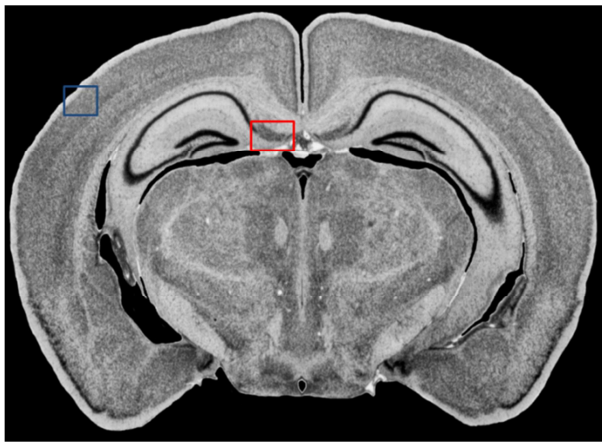
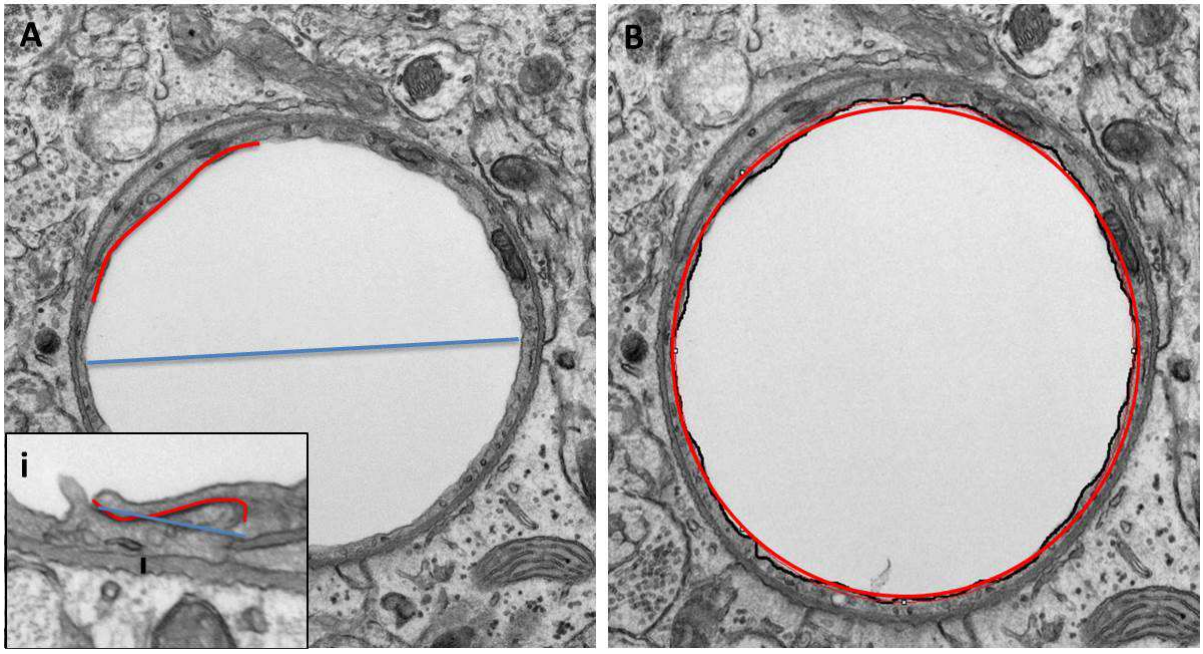
Supplementary Methods

Image analysis for electron microscopy

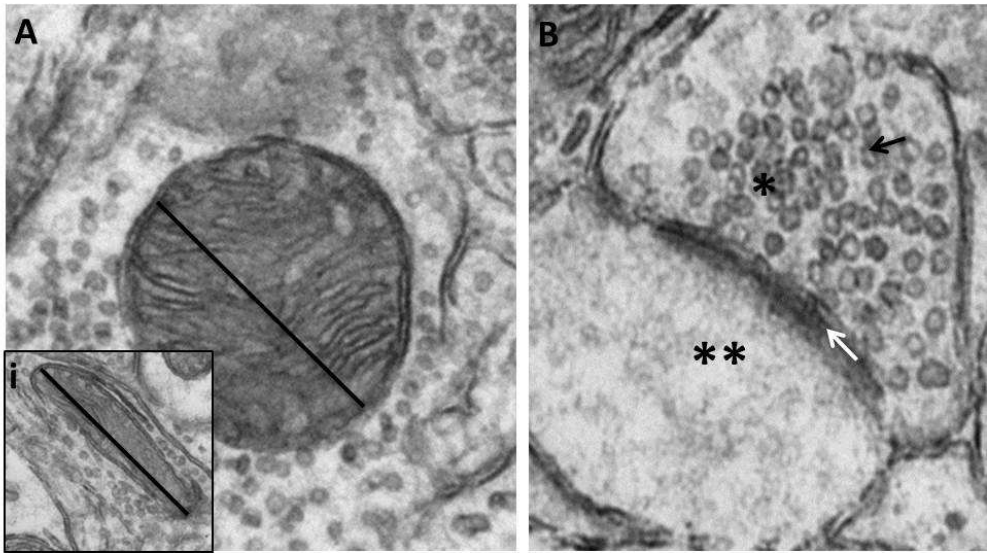
For each sample, 10 electron micrographs of capillaries and neuropil were taken from the subiculum of the hippocampus, and the somatosensory cortex containing layers 1 to 3 (Supplementary Figure 1) and analysed using ImageJ (NIH, USA). On each micrograph the following endothelial measurements were made: basal lamina thickness (nm), vessel lumen diameter (µm), vessel lumen roughness, percentage of pericyte coverage, tight junction tortuosity and number of endothelial vesicles per µm of vessel wall (Supplementary Figure 2). For neuronal views (63 μm^2) mitochondrial length and synaptic number were measured (Supplementary Figure 3).



Supplementary Figure 1. Microdissection sites taken for electron microscopy. The insets indicate the areas taken from each sample: the subiculum of the hippocampus (red) and the primary somatosensory cortex (blue).



Supplementary Figure 2. Endothelial measurements in electron micrographs. (A) Lumen diameter: the vessel lumen (blue line) was calculated by taking the area of the lumen, using the convex hull tool in ImageJ, and calculating the diameter by using the formula of the circle area, being that the diameter will be twice the radius (diameter = 2 x $\sqrt{area \div \pi}$). **Pericyte coverage**: calculated as the percentage of the lumen that was covered by a pericyte (red line), therefore the length of the lumen covered by the pericyte cell body was calculated and divided by the total perimeter of the vessel. **(i) Tight junction tortuosity (inset):** calculated as the ratio between the measured length of the tight junction from the luminal side to the basal lamina (red line) and the length of a straight line (blue line) drawn directly from one side to another of the tight junction. **Basal lamina thickness (inset):** the mean thickness of the basal lamina (black line) was calculated by measuring 3 equidistant locations. **(B) Vessel lumen roughness:** calculated by the ratio of the lumen perimeter (black) to an ideal perimeter if the blood vessel was perfectly smooth (red), this was quantified by using the convex hull tool in ImageJ. Ratios greater than 1 indicate vessel imperfections, while a value of 1 suggests a perfectly smooth vessel.



Supplementary Figure 3. Mitochondrial length and identification of synapses. (A) Mitochondrial length: mitochondrial length was calculated by measuring the major length (black line) of the mitochondria from one side to another using ImageJ as shown for normal and rounded mitochondria (A) and in elongated mitochondria (i). **(B) Synapses:** synapses were identified as an electron dense synaptic cleft (white arrow) between a presynaptic neurone (star) containing synaptic vesicles (black arrow), and the postsynaptic neurone (two stars).

Chapter 5

Attenuation of obese phenotype by resveratrol does not improve memory in high-fat diet fed mice

Isaura Martins, Helen Parker, Stuart M. Allan, Catherine B. Lawrence

Attenuation of obese phenotype by resveratrol does not improve memory in high-fat diet fed mice

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Keywords: obesity; Alzheimer’s disease, AD; control non-transgenic, Non-Tg; triple-transgenic AD, 3xTgAD; high-fat diet; obesity; resveratrol, RSV; memory; behaviour; adipose tissue.

Abstract

Obesity and neurodegenerative diseases are increasing worldwide, and it is known that obesity itself is a risk factor for developing dementia later in life, in particular Alzheimer's disease (AD). Obesity and high-fat diets can affect memory and high-fat diets can also exacerbate cognitive impairment in AD mouse models. However, whether the detrimental effect of a high-fat diet on memory is dependent on the development of obesity is unknown.

The aim of the present study was to evaluate whether the effect of a high-fat diet on memory in mice was due to obesity by using resveratrol (RSV), a plant based polyphenol that has been shown to have anti-obesogenic properties. Female non-transgenic (Non-Tg) control and triple transgenic AD mice (3xTgAD) were fed either a control or a high-fat diet with or without RSV treatment (5mg/kg/day) for 12 months. A high-fat diet impaired memory in Non-Tg but had no effect in 3xTgAD mice. In high-fat fed Non-Tg control mice, RSV reduced body weight, white adipose tissue (WAT), brown adipose tissue (BAT) and liver weight but had no effect on memory impairment. In 3xTgAD mice, RSV had no effect on the increase in body weight or WAT induced by high-fat diet, but reduced BAT weight. At 12 months, RSV improved memory performance in control-fed but not in high-fat fed 3xTgAD mice when assessed using a smell recognition test.

These findings suggest that the negative effect of a high-fat diet on memory in Non-Tg mice is independent of obesity. Furthermore, as RSV improved memory deficits in control-fed 3xTgAD mice, the mechanisms underlying the detrimental effects of a high-fat diet on memory are likely to be different to those causing memory impairment in AD.

Introduction

The overconsumption of “Westernised” diets, which are rich in fatty acids, is associated with a rise in obesity. Epidemiological studies suggest that obesity is a major risk factor for cognitive impairment, in particular for the development of Alzheimer’s disease (AD) [1-10] and in countries where consumption of high-fat diets is higher, the prevalence of AD is greater [11, 12].

Both obesity and AD are associated with memory impairment. AD is characterised by progressive memory loss, due to cell death and decreased hippocampal volume [13]. The hippocampus appears to be sensitive to a change in dietary components, in particular to saturated fatty acids (SFAs), and high-fat diets affect hippocampal-dependent memory and hippocampal integrity in cognitively normal rodents [14-24]. In addition, high-fat feeding exacerbates memory impairment in mouse models of AD [25-31]. However, mechanism underlying the detrimental effect of HFD on memory and whether it is dependent on the obese phenotype are unknown.

Resveratrol (3,5,4’-trihydroxy-*trans*-stilbene; RSV), a natural polyphenol produced by many plants and present in fruits such as grapes and berries [32], protects against oxidative stress, inflammation, cardiovascular diseases and cancer [32-35]. Recently, experimental studies have shown that RSV can have neuroprotective effects in AD [36-46]. Indeed, epidemiological studies have demonstrated that moderate consumption of red wine, that contains RSV, is associated with lower incidence of dementia and AD [47]. RSV has been shown to attenuate amyloid-β (Aβ)-induced toxicity [39, 48], promote Aβ clearance in *in vitro* and *in vivo* studies [42, 46], reduce senile plaques [40] and improve spatial memory function in a mouse model of AD [36].

RSV is also anti-obesogenic and protects against the effects of a high-fat diet in rodents by increasing insulin sensitivity, reducing internal adipose tissues weight, reducing body weight and down-regulating adipogenesis [49-56]. A recent study has reported that a high dose of RSV (200 mg/kg/day) improves memory deficits in mice fed with a high-fat diet without reducing in body weight [51]. Studies have also shown that RSV can enhance cognitive performance [57-62] and attenuate memory deficits associated with AD [39, 43, 63]. However, whether a reduction in obesity in response to RSV can affect memory impairment induced by high-fat diet remains unclear.

The majority of studies showing that RSV can reduce body weight in response to a high-fat diet have used low doses of RSV (0.5 to 30 mg/kg/day). Therefore, the aim of this study was to investigate if long-term administration of low doses of RSV can reverse the obesity phenotype, and whether this can affect the memory deficits associated with a high-fat diet. For that purpose we choose a low dose of RSV (5mg/kg/day) that has been demonstrated to partially mimic the effects of caloric restriction (CR) in mice in the absence of metabolic and endocrine disturbances or reduced caloric intake [64]. Mimicking CR would potentially lead to a reduction of body weight in high-fat fed mice and would also be relevant in AD as it has been demonstrated that CR attenuates AD pathology in mice, by reducing amyloid deposition in animal models [65-67] and to improves memory performance in the triple transgenic model (3xTgAD) [68].

Therefore, groups of female control non-transgenic (Non-Tg) and 3xTgAD mice were kept on either a control or a high-fat diet with or without oral RSV treatment. The obese phenotype was monitored and memory assessed using several behaviour tests after 12 months.

Methods

Animals and drug administration

An in-house colony of 3xTgAD mice, expressing mutant $PS1_{M146V}$, APP_{SWE} and Tau_{P301L}, and Non-Tg controls, on a C57BL6 x 129SV background, was established from breeding pairs originally supplied by Frank LaFerla (Irvine, CA, USA) [69]. Mice were kept in standard housing conditions (humidity 50%-60%, temperature 21 ± 1˚C, 12:12 hour light-dark) and given *ad libitum* access to food (BK001, Special Diets Services, UK) and water. At 5 weeks of age groups of female Non-Tg and 3xTgAD mice were randomly separated into four groups (n = 6-7/group) and allocated to either a control (12% energy from fat, 5% fat content by weight, 0.78% saturated fatty acids, 58G7, Test Diets) or high-fat diet (60% energy from fat, 35% fat content by weight, 13% saturated fatty acids, 58G9, Test Diets) with and without RSV for 12 months. RSV in 0.1% ethanol (5 mg/kg/day of 99% trans-RSV (Bulk Powders, UK)) was supplemented in the drinking water and was made up fresh every 3-4 days. Vehicle groups received water containing 0.1% ethanol without RSV. Body weight

and water intake was monitored weekly over 12 months and a 24 h average water intake was calculated per mouse per group. The concentration of RSV was then adjusted according to any change in body weight and water intake to maintain a dose of 5mg/kg/day. All animal experiments were carried out in accordance with the United Kingdom Animals (Scientific Procedures) Act 1986 and approved by the Home Office and the local Animal Ethical Review Group, University of Manchester.

Behaviour

After 6 and 12 months on diet and RSV supplementation, behaviour was assessed in female Non-Tg control and 3xTgAD mice using the open-field, Y-maze spontaneous alternation, novel object recognition and smell recognition tests. To decrease anxiety, all animals were allowed to habituate to the test room for 30 minutes prior to all behavioural tests. All experiments were performed between 10am and 5pm in the same testing room. Each test was performed on a different day and the animal order was randomised between groups. All apparatus were cleaned between animals and tests with 70% ethanol. Analysis of all behavioural data was performed blinded to genotype, diet and treatment.

Open-field test

General motor function and exploratory activity was assessed in the open-field. Mice were placed in an unfamiliar environment, which consisted of a square opaque Perspex box (45 cm x 45 cm x 30cm). The animal was positioned in the centre of the arena and allowed to explore for 5 minutes. Behaviour was recorded with a video camera and Any-Maze Tracking software (Stoelting Co, Dublin, Ireland) was used to track movement. Total time moving was measured and % time moving calculated.

Y-maze spontaneous alternation test

Short-term working memory was assessed using the Y-maze spontaneous alternation test. A black opaque Perspex Y-shaped maze with three arms (A, B and C) was used and a visual cue was

located at each end of the arm (arm dimensions; 15 cm x 10 cm x 10 cm). Each animal was placed into the maze facing the end of arm A and allowed to explore for 8 minutes. The number of arm entries (when the entire body of the animal was inside the arm space) made by each animal was recorded. Spontaneous alternation was defined as three consecutive entries in different arms (triplets) in a random order and the percentage number of alternations between arms was calculated as described previously [25].

Novel object and smell recognition tests

To evaluate recognition memory, based on the spontaneous tendency of the animal to spend more time examining a novel smell or object compared to a familiar one [70], smell recognition and novel object recognition tests were used as described previously [25]. Briefly, for both tests, all animals were allowed to habituate to a black opaque polycarbonate circular arena (diameter, 30 cm x height, 21 cm) for 5 minutes over 2 days. On the third day, mice were placed in the centre of the arena and allowed to explore 2 identical objects, for novel object recognition test, or 2 identical scented balls, for smell recognition test, for 10 minutes (phase 1). Mice were then removed and one of the objects was replaced with a novel object that varied in shape and colour, or for the smell recognition test one of the scented balls was replaced by a ball containing a novel scent. After a delay of 1 hour for novel object recognition or 3 minutes for smell recognition, mice were placed back into the arena and allowed to explore for 4 minutes (phase 2). The scented balls (Chad Valley, UK) were filled with cotton wool and 0.5 ml of scent (orange, lemon, vanilla, almond, peppermint or eucalyptus, Dr Oetker Ltd, UK) was distributed into the balls via small holes. All experiments were recorded with a camera (Sanyo Xacti VPC-C4, SANYO Fisher, CA, USA) and MP4 video-clips were converted to an AVI format using Pazera MP4 to AVI converter 1.3 (Pazera-Software, Poland). The time (seconds) spent exploring the objects was measured using SR Ethovision XT 8.5 (Noldus, Wageningen, The Netherlands) whilst time spent exploring the scented balls was calculated manually by observing each individual video. Exploration was defined as the amount of time that the animals spent with their nose within 2 cm in the direction of the smell/object. The percentage of time spent exploring the familiar and novel object/smell was calculated for each phase of the test.

Tissue collection and glucose measurement

Mice were terminally anesthetised with 3.5% isoflurane (30% O_2 and 70% N_2O) and blood was taken from the heart using 3.8% sodium citrate as an anticoagulant. To remove all blood, animals were then perfused transcardially with 0.9% saline. Total gonadal white adipose tissue (WAT), interscapular brown adipose tissue (BAT), liver and spleen were dissected and weighed. Non-fasting blood glucose was measured in cardiac blood by an Accu-Chek Diabetes kit (Roche Diagnostics, Indianapolis, IN, USA).

Statistical analyses

All data are expressed as the mean ± standard error of the mean (SEM). Planned comparisons were to test the effect of RSV on high-fat diet induced changes in Non-Tg control and 3xTgAD mice and not to compare the effects between genotypes. No statistical analysis was performed on estimated average water intake per mouse, as these data were derived from group housed animals. Body and organ weights, glucose, and data from the open-field and Y-maze spontaneous alternation tests were analysed using a two-way analysis of variance (ANOVA) followed by Bonferroni´s post-hoc test using SigmaPlot (Systat Software, Inc., San Jose, CA, USA). For the smell recognition and novel object tests a Student's *t* test was used (GraphPad Prism 6, GraphPad Software, Inc., CA, USA). Statistical significance was taken when $p < 0.05$.

Results

RSV prevented the increase in body weight induced by high-fat diet in control Non-Tg but not 3xTgAD mice

Over a 12-month period, Non-Tg control and 3xTgAD mice had different body weight and water intake profiles in response to a high-fat diet and RSV (Figure 1A-D). In Non-Tg control mice, a high-fat diet led to an increase in body weight after 4.5 months (Figure 1A) but an increase in body weight was not observed until after 5 months in 3xTgAD mice (Figure 1C). In response to RSV, body weight did not change in control-fed mice of both genotypes when compared to control-fed mice treated with vehicle (Figure 1A, C). A high-fat diet failed to increase body weight over 12 months in Non-Tg mice treated with RSV as no significant difference in body weight was observed when compared to control fed Non-Tg mice (either treated with vehicle or RSV) (Figure 1A). From 9 months there was a significant reduction in body weight in high-fat fed Non-Tg mice treated with RSV when compared to high-fat fed Non-Tg mice treated with vehicle (Figure 1A). No effect of RSV on body weight was observed in high-fat diet fed 3xTgAD mice as a significant increase in body weight was observed after 5 months in this group of mice when compared to control fed mice treated with RSV (Figure 1C).

Although no statistical analysis was made on the estimated average weekly water intake per mouse, there was a clear difference in water intake profiles throughout the 12 months between control and high-fat diet Non-Tg mice (Figure 1B). Both high-fat fed Non-Tg groups, treated with vehicle or RSV, drank less than their respective groups fed on a control diet (44% and 45% respectively) (Figure 1B). This effect of a high-fat diet on water intake was not observed in 3xTgAD mice, where the average water intake was similar between diets (in vehicle or RSV) during the 12-month monitoring period (Figure 1D).

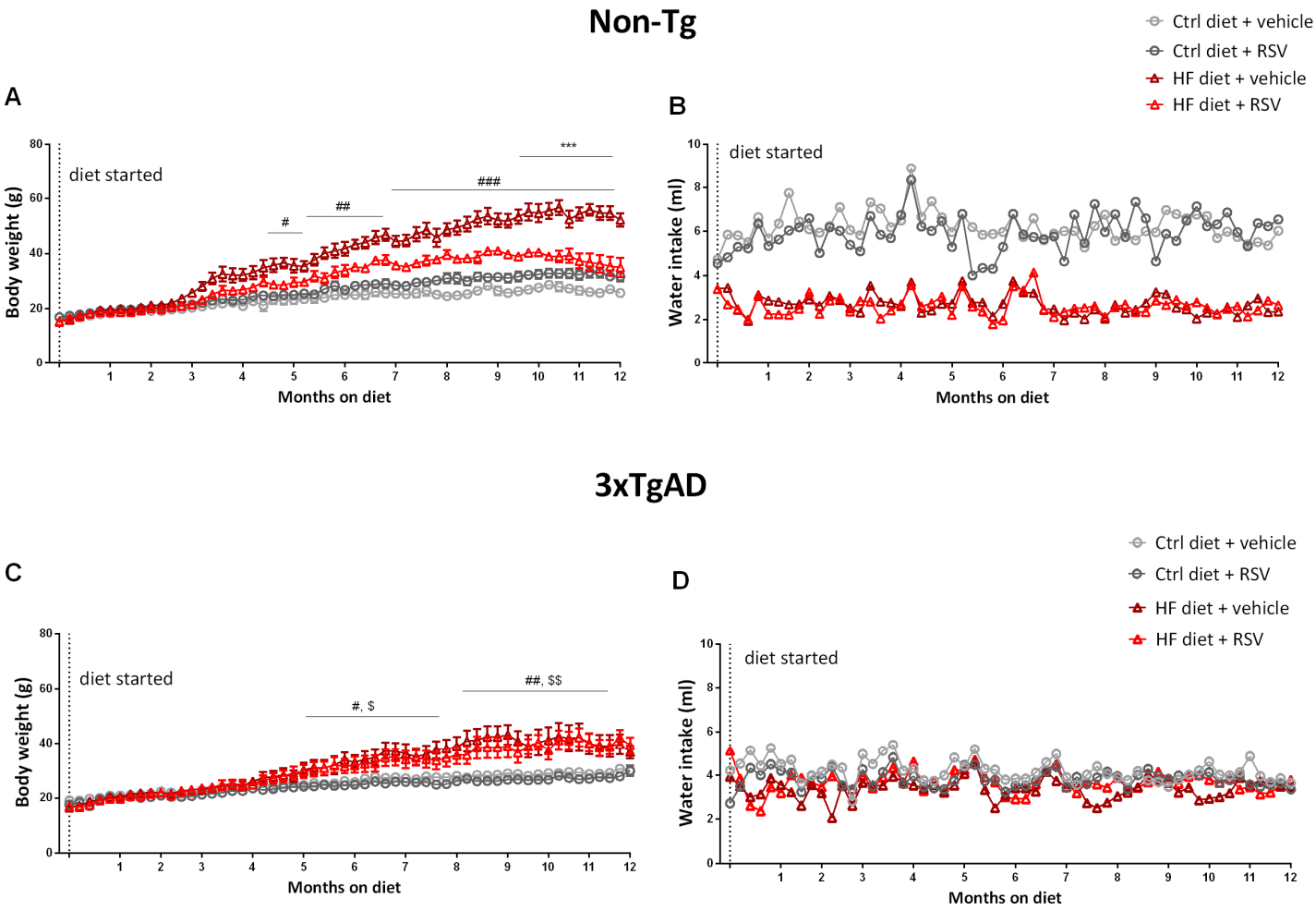

Figure 1. RSV prevented the increase in body weight induced by high-fat diet in control Non-Tg but not 3xTgAD mice. Groups of 5-week-old female Non-Tg and 3xTgAD mice were maintained on a high-fat diet and treated with vehicle (HF diet & vehicle) or RSV (HF diet & RSV) at 5 mg/kg/day, or on a control diet and treated with vehicle (Ctrl diet & vehicle) or RSV (Ctrl diet & RSV) for 12 months. Body weight (A, C) and water intake (B, D) were assessed weekly until 12 months. Body weight is represented as mean ± SEM. Water intake is represented as the average water intake per mouse derived from group housed mice. n = 6-7/group. # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ for high-fat diet & vehicle versus control diet & vehicle, $ $p < 0.05$, $$ $p < 0.01$ for high-fat diet & RSV versus control diet & RSV, *** $p < 0.001$ for high-fat diet & vehicle versus high-fat diet & RSV. Two-way ANOVA with Bonferroni's post hoc analysis.

A high-fat diet and RSV had no effect on glucose levels in both Non-Tg control and 3xTgAD mice

At 12 months no change in non-fasting blood glucose levels was observed in either Non-Tg control (Figure 2A) or 3xTgAD (Figure 2B) mice fed a high-fat diet or with RSV supplementation.

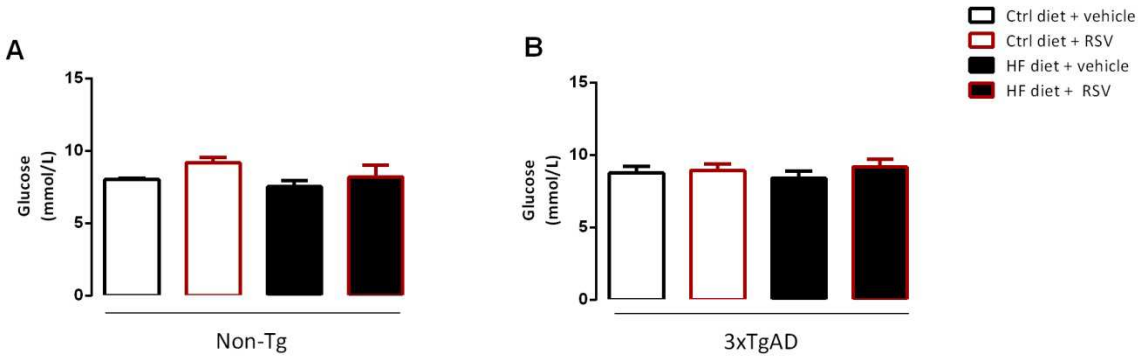

Figure 2. Glucose levels in female Non-Tg and 3xTgAD mice were unchanged with high-fat diet and RSV treatement. Groups of 5-week-old female Non-Tg and 3xTgAD mice were maintained on a high-fat diet and treated with vehicle (HF diet & vehicle) or RSV (HF diet & RSV) at 5 mg/kg/day, or on a control diet and treated with vehicle (Ctrl diet & vehicle) or RSV (Ctrl diet & RSV) for 12 months. Blood glucose (mmol/l) levels were measured after 12 months. Data are mean ± SEM, n = 6-7/group.

RSV attenuated the obese phenotype induced by high-fat diet in female Non-Tg mice

In vehicle-treated Non-Tg control mice, a high-fat diet increased body-weight (Figure 3A), WAT (Figure 3C), BAT (Figure 3E) and liver weight (Figure 3G), whilst an increase in body-weight (Figure3B), WAT (Figure 3D) and BAT (Figure 3F) was seen in vehicle treated 3xTgAD mice. In both Non-Tg groups a high-fat diet led to paler and fatty livers and RSV treatement alter this appearance. RSV supplementation reversed this increase in body weight (Figure 3A) and organ weight in Non-Tg control mice (Figure 3C, E, G) but only reduced BAT weight in 3xTgAD mice (Figure 3F). RSV supplementation increased BAT and liver weight in control fed Non-Tg mice (Figure 3E, G), with no effect in control fed 3xTgAD mice. In control fed Non-Tg mice the increase in liver weight was observed with no differences in the normal liver appearance. Spleen weight was unchanged with high-fat diet or RSV in both genotypes (Figure 3I, J).

RSV had no effect on memory deficits in high-fat fed Non-Tg mice but improved memory deficits in 3xTgAD mice in smell recognition test

In female Non-Tg and 3xTgAD mice behaviour was assessed after 6 and 12 months on a high-fat diet with or without RSV treatement. In the open-field test, no effect of diet or RSV supplementation was observed on % time moving in both genotypes at any time assessed (Figure 4A-D). In order to assess hippocampal-dependent memory, Y-maze spontenous alternation, novel object recognition and smell recognition tests were used. In the Y-maze spontaneous alternation test, neither a high-fat diet or RSV had an effect on % alternation in both Non-Tg and 3xTgAD mice at 6 or 12 months (Figure 5A-D).

In both novel object and smell recognition tests no difference in exploration time between the two identical objects or smells during phase 1 was observed in all groups of mice (data not shown). In the novel object recognition test all Non-Tg mice fed a control diet had intact memory, as all mice spent significantly ($p<0.05$) more time exploring the novel object, whilst Non-Tg mice fed a high-fat diet had significant impairments after 6 months (Figure 6A). RSV treatment was not able to reverse the effect of high-fat diet on memory in the Non-Tg control mice (Figure 6A). In 3xTgAD mice, all groups presented memory deficits in the novel object recognition at 6 months (Figure 6B), since no significant difference in time exploring both objects were observed. RSV had no effect on the memory deficits in either control or high-fat fed 3xTgAD mice.

In the smell recogntion test, there was no effect of a high-fat diet on memory in Non-Tg control mice after 6 or 12 months, and groups were able to perform the test (Figure 7A, C). In 3xTgAD mice after 6 months all groups showed impaired memory (Figure 7B), however after 12 months, RSV supplementation was able to reverse memory deficits in control fed 3xTgAD mice but had no efect in high-fat fed mice (Figure 7D).

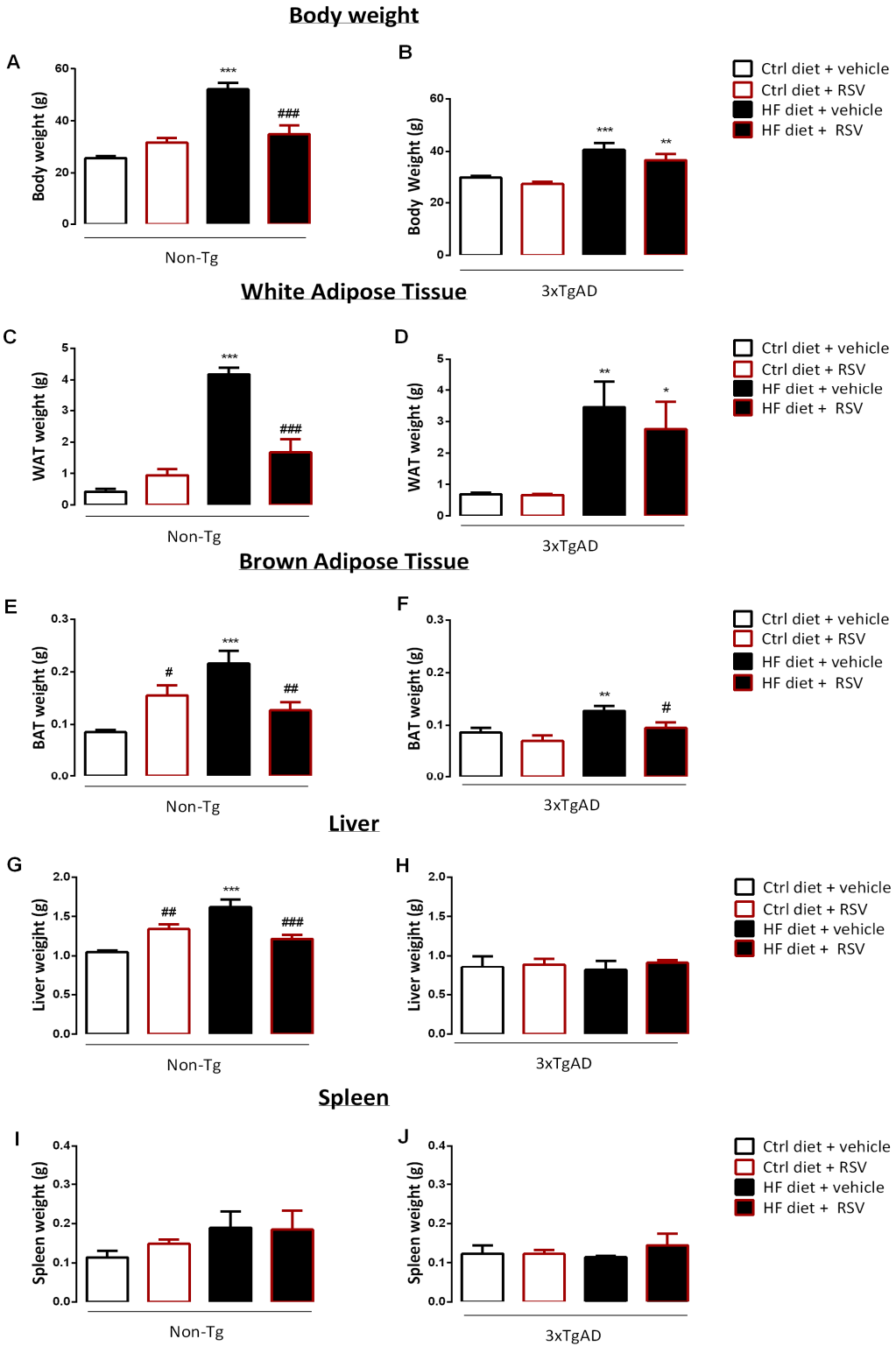

Figure 3. RSV attenuated the obese phenotype induced by high-fat diet in female Non-Tg control mice. Groups of 5-week-old female Non-Tg and 3xTgAD mice were maintained on a high-fat diet and treated with vehicle (HF diet & vehicle) or RSV (HF diet & RSV) at 5 mg/kg/day, or on a control diet treated with vehicle (Ctrl diet & vehicle) or RSV (Ctrl diet & RSV) for 12 months. Body, white adipose tissue (WAT), brown adipose

tissue (BAT), spleen and liver weight were measured after 12 months. Data are mean ± SEM, n = 6-7/group. * p < 0.05, ** p < 0.01, *** p < 0.001 for high-fat diet versus control diet on the same treatment. # p < 0.05, ## p < 0.01, ### p < 0.001 for RSV versus vehicle on the same diet. Two-way ANOVA with Bonferroni's post hoc analysis.

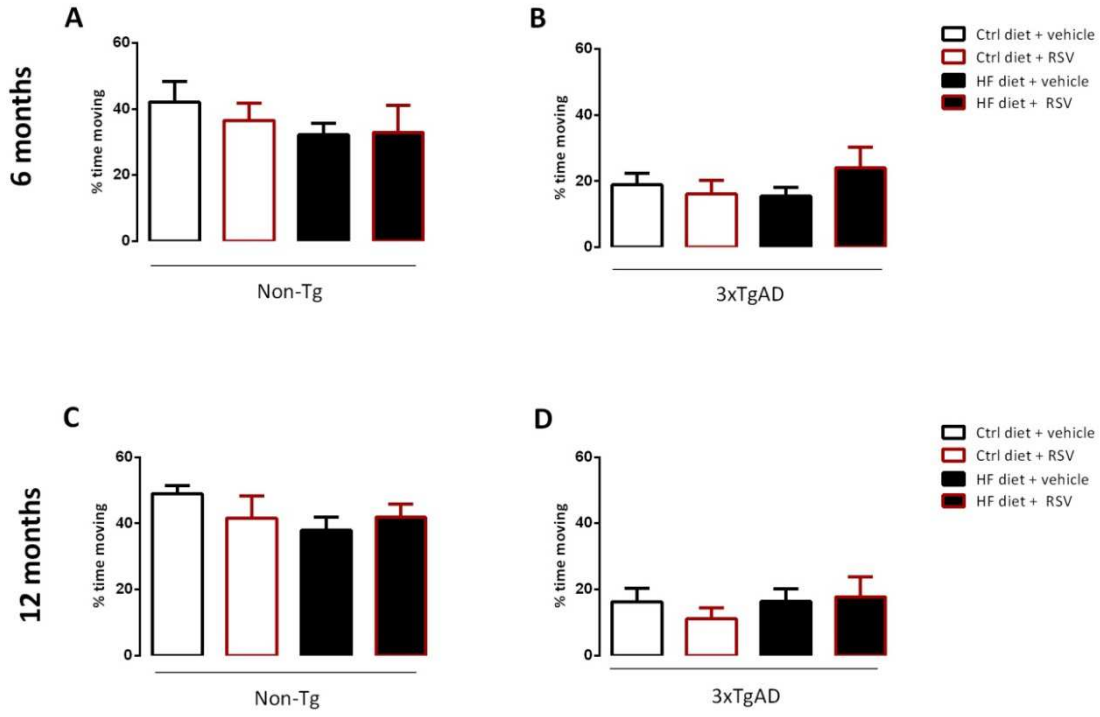

Figure 4. A high-fat diet had no effect on movement in the open-field test in female Non-Tg control and 3xTgAD mice. Groups of 5-week-old female Non-Tg and 3xTgAD mice were maintained on a high-fat diet and treated with vehicle (HF diet & vehicle) or RSV (HF diet & RSV) at 5 mg/kg/day, or on a control diet and treated with vehicle (Ctrl diet & vehicle) or RSV (Ctrl diet & RSV) for 12 months. Spontaneous exploratory behaviour was assessed in the open-field test at 6 and 12 months. Data are mean ± SEM, n = 6-7/group.

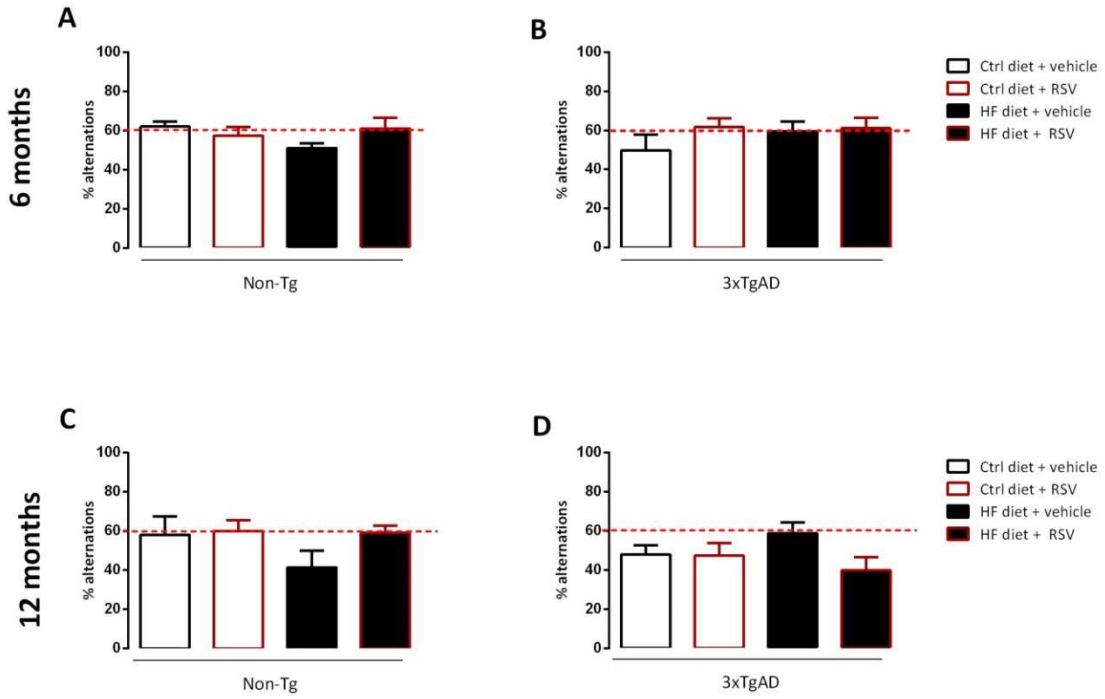

Figure 5. RSV and a high-fat diet did not affect memory in female Non-Tg or 3xTgAD mice in the Y-maze spontaneous alternation test. Groups of 5-week-old female Non-Tg and 3xTgAD mice were maintained on a high-fat diet and treated with vehicle (HF diet & vehicle) or RSV (HF diet & RSV) at 5 mg/kg/day, or on a control diet and treated with vehicle (Ctrl diet & vehicle) or RSV (Ctrl diet & RSV) for 12 months. Memory was assessed in the Y-maze spontaneous alternation test after 6 and 12 months. No effect of a high-fat diet or RSV treatment was observed in Non-Tg or 3xTgAD mice. Data are mean ± SEM, n = 6-7/group.

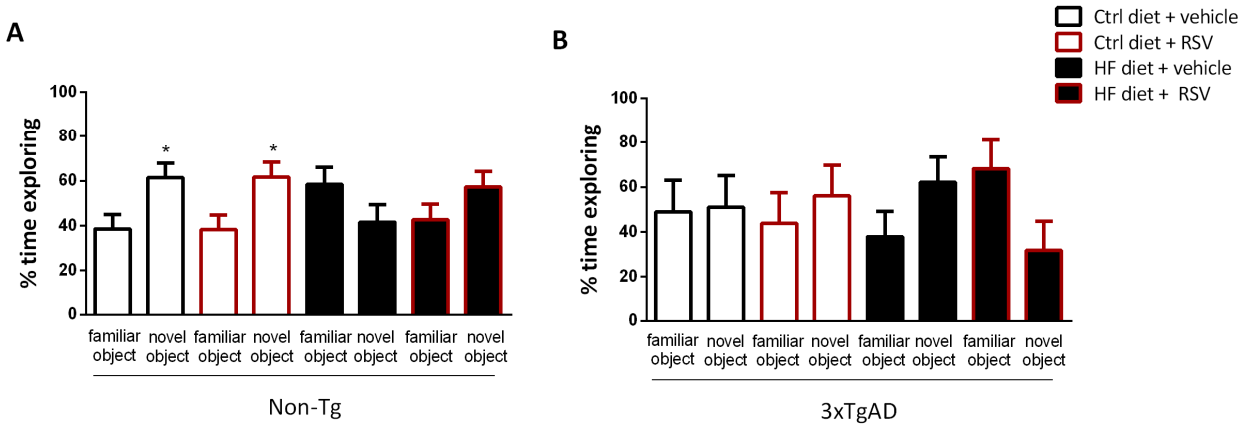

Figure 6. RSV had no effect on memory impairment observed in high-fat fed female Non-Tg or in 3xTgAD mice after 6 months in the novel object recognition test. Groups of 5-week-old female Non-Tg and 3xTgAD mice were maintained on a high-fat diet and treated with vehicle (HF diet & vehicle) or RSV (HF diet & RSV) at 5 mg/kg/day, or on a control diet treated with vehicle (Ctrl diet & vehicle) or RSV (Ctrl diet & RSV). Memory was assessed in the novel object recognition test after 6 months. A high-fat diet impaired memory after 6 months in Non-Tg mice as no difference exploring the novel versus familiar object was observed in high-fat fed Non-Tg mice. RSV had no effect on this impairment as high-fat fed Non-Tg mice treated with RSV spent the same amount of time exploring the novel and familiar object. 3xTgAD mice fed a control or high-fat diet had memory impairment. No effect of RSV on memory was seen in 3xTgAD mice on control or high-fat diet. Data are mean ± SEM, n = 6-7/group. * $p < 0.05$ versus familiar object. Student's *t* test.

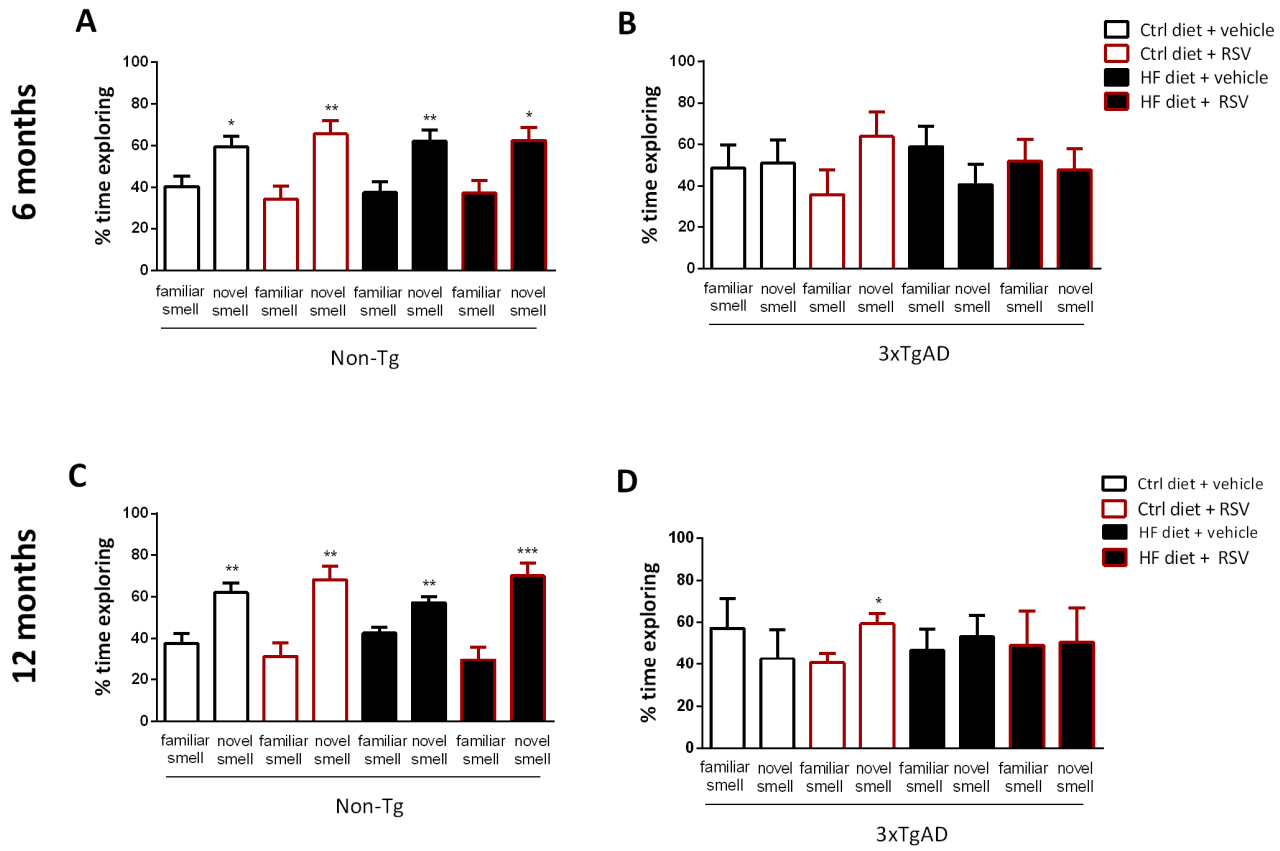

Figure 7. RSV improved memory in female 3xTgAD mice fed on a control diet after 12 months in smell recognition test Groups of 5-week-old female Non-Tg and 3xTgAD mice were maintained on a high-fat diet and treated with vehicle (HF diet & vehicle) or RSV (HF diet & RSV) at 5 mg/kg/day, or on a control diet and treated with vehicle (Ctrl diet & vehicle) or RSV (Ctrl diet & RSV) for 12 months. Memory was assessed by the smell recognition test after 6 and 12 months. A high-fat diet had no effect on memory in Non-Tg at both time points. Memory was impaired in both control and high-fat fed 3xTgAD mice at 6 and 12 months. RSV reversed the memory deficit at 12 months in control fed 3xTgAD mice but had no effect in high-fat fed mice. Data are mean ± SEM, n = 6-7/group. * $p < 0.05$, ** $p < 0.01$. *** $P < 0.001$ versus familiar smell. Student's *t* test.

Discussion

High-fat diets are known to have detrimental effects on cognition in both cognitively normal rodents [19-25, 71-73] and in AD mouse models [25-31]. However, the molecular pathways that underlie the effect of high-fat diet on memory remain unclear. Moreover, is known that fat transplantation from genetically obese mice into control mice induces memory impairment [22].

RSV has anti-obesogenic properties and can improve cognition in high-fat fed rodents [51] and in rodent models of AD [36-46].]. However, whether these beneficial effects of RSV on memory after high-fat feeding are due to a reduction in obesity are unknown. The present study was the first to evaluate if the effect of a high-fat diet on memory in mice was due to obesity by testing if cognitive performance can be improved after RSV treatment.

Our results demonstrate that in Non-Tg mice a high-fat diet led to a decrease in water intake, an increase in body, fat (WAT and BAT) and liver weight, and impaired memory. RSV treatment reversed the obese phenotype but had no effect on the memory deficits in high-fat fed Non-Tg mice. In contrast, RSV had no effect on body weight and adiposity (WAT) or memory impairment in high-fat fed 3xTgAD mice, but improved memory in control fed 3xTgAD mice. These data suggest that reducing obesity does not improve the detrimental effect of high-fat diet on memory.

The anti-obesogenic properties of RSV are widely reported [50-52, 56, 71]. Anti-obesogenic effects are proposed to be due to a reduction in body weight gain by increasing mitochondria biogenesis and physical endurance [72], improvements in metabolic profile by modulating adipose tissue function [52, 73] and a reduction in fat accumulation [49, 72]. The reduction in body fat by RSV might also be mediated by a reduction in fatty acid uptake from circulating triacylglycerols and from *de novo* lipogenesis [73].

RSV can also prevent the development of enlarged and fatty liver in response to a high-fat diet in mice [49, 74], which supports findings here in Non-Tg mice. In rats fed a high-fat diet, RSV decreases liver lipogenic activity [73, 75] and prevents fatty liver disease by increasing hepatic mitochondria [76]. Our results also showed that RSV increased liver weight in Non-Tg mice on a control diet without the appearance of a fatty and pale liver. This effect on liver could be due to RSV acting as a mild biological stressor, inducing a mild state of inflammation, which could potentially

contribute to an increase in the liver weight, however this needs to be investigated in more detail in future experiments.

As expected, high-fat diet increased fat content in both Non-Tg and 3xTgAD mice. In high-fat fed Non-Tg mice RSV has able to reduce adiposity, an effect that was not seen in high-fat fed 3xTgAD mice. These data suggest that 3xTgAD mice respond differently to RSV treatment, which might be due to the metabolic differences reported previously in these mice [77].

Obesity is characterised by an increase in WAT mass, however BAT is inversely correlated with body mass index (BMI) in humans [78-80]. In mice, obesity and high-fat diets increase both BAT and WAT in some studies [81-83]. In the present study a high-fat diet increased both BAT in both Non-Tg and 3xTgAD. RSV reduced the increase in interscapular BAT and WAT weight induced by high-fat diet in Non-Tg and 3xTgAD mice but only WAT in Non-Tg mice. It is known that BAT is capable of transdifferentiating into WAT and vice versa to meet the energy demands [84]. Therefore is not surprising that if RSV is able to reduce WAT is also has an effect in BAT. However, RSV increased interscapular BAT weight in control fed Non-Tg mice without affecting total body weight. Although experimental data on BAT remains limited, it is known that with age BAT can be impaired in rodents, compromising its function and thermogenic capacity. In addition, this age-associated decline of thermogenesis has been linked to BAT atrophy [85]. Finally, it is known that in humans BAT plays an important role in energy homeostasis and that large amounts of BAT are associated with a decreased risk for developing obesity and obesity related-disorders in humans [86]. Therefore, our data could indicate that an increase of BAT by RSV administration in control fed Non-Tg mice could be a protective mechanism to enhance thermogenesis and improve energy homeostasis. However, other BAT depots should be assessed in future studies to evaluate the effect of RSV on BAT in different locations.

Previous studies have shown that RSV is able to reduce hyperglycemia induced by high-fat diet/obesity [87-89], however in our study a high-fat diet had no effect on non-fasting blood glucose levels in either Non-Tg control or 3xTgAD mice, therefore memory deficits are unlikely to be due to hyperglycemia.

Water intake in response to a high-fat diet was different between Non-Tg and 3xTgAD mice. A high-fat diet reduced water intake in Non-Tg mice with or without RSV treatment. This effect may be due to differences in caloric intake during a high-fat diet which might influence the amount of water intake or due to pellet consistency, however further studies involving analysis of food intake should be done to confirm this. There was no difference in water intake in response to a high-fat diet in 3xTgAD mice, which suggests that these mice respond differently to a high-fat diet.

In the present study a high-fat diet had no effect on behaviour or memory in 3xTgAD mice. Although we have previously demonstrated that a high-fat diet can increase the memory deficits in male 3xTgAD mice [25], the present study performed in female 3xTgAD mice does not confirm that. However, since control fed 3xTgAD mice already presented with memory deficits after 6 months on diet in both the novel object recognition and smell recognition tests, a further effect of high-fat diet could not be observed using these particular behavioural tests. RSV treatment had no effect on the memory deficits in high-fat fed 3xTgAD mice and had no effect on body weight or adiposity. In contrast, RSV attenuated the memory deficits in control fed 3xTgAD mice when assessed using the smell recognition test after 12 months. These data suggest that the consumption of a high-fat diet in 3xTgAD mice impaired the ability of RSV to improve memory deficits in these mice.

A high-fat diet had a negative effect on memory in Non-Tg control mice after 6 months when assessed by novel object recognition. These data are supported by previous studies where high-fat diets impair memory in mice [16-18, 21, 22, 25, 90]. However, although RSV was able to reverse the obese phenotype in high-fat fed Non-Tg mice, at 6 months it did not improve memory deficits, suggesting that memory impairment induced by high-fat diet in Non-Tg mice is independent of obesity. The only study to date that has demonstrated a beneficial effect of RSV on memory deficits induced by high-fat diet, showed no effect of RSV on weight gain [51]. Jeon et al have shown that RSV (200 mg/kg/day) had no effect on body weight in high-fat fed mice but was able to improve cognition by improving peripheral inflammation and insulin sensitivity [51]. In contrast, results presented here show that a reduction in adiposity and body weight is not sufficient to attenuate the detrimental effect of a high-fat diet on cognition in Non-Tg mice. Memory deficits induced by high-fat diets are also associated with increased hippocampal inflammation [51], disruption of the BBB [14,

56, 91, 92] and insulin resistance [21, 93-95]. Therefore, it might be that the dose of RSV used here, very similar to the amount of RSV in a glass of red wine (0.2 – 2 mg of RSV), was sufficient to induce a reduction in the obese phenotype (adiposity) in Non-Tg mice but not to exert an effect on insulin sensitivity and inflammation and other molecular events that could affect cognitive performance, such as oxidative stress.

In conclusion, our data suggest that the negative effect of a high-fat diet on memory is independent of obese phenotype as a low dose of RSV reduced obesity but failed to improve memory deficits in Non-Tg mice. Furthermore, as RSV improved memory deficits in 3xTgAD mice on a control diet, the mechanisms underlying cognitive impairment in AD are likely to be different to those involved in the detrimental effects of a high-fat diet on memory.

References

1. Naderali, E.K., S.H. Ratcliffe, and M.C. Dale, *Review: Obesity and Alzheimer's Disease: A Link Between Body Weight and Cognitive Function in Old Age.* American Journal of Alzheimer's Disease and Other Dementias, 2009. **24**(6): p. 445-449.
2. Elias, M.F., et al., *Obesity, diabetes and cognitive deficit: The Framingham Heart Study.* Neurobiology of Aging, 2005. **26**(1, Supplement): p. 11-16.
3. Anstey, K.J., et al., *The influence of smoking, sedentary lifestyle and obesity on cognitive impairment-free life expectancy.* International Journal of Epidemiology, 2014.
4. Kanoski, S.E. and T.L. Davidson, *Western diet consumption and cognitive impairment: Links to hippocampal dysfunction and obesity.* Physiology & Behavior, 2011. **103**(1): p. 59-68.
5. Xu, W.L., et al., *Midlife overweight and obesity increase late-life dementia risk: a population-based twin study. .* Neurology, 2011. **76**: p.:1568–1574.
6. Fitzpatrick, A.L., et al., *Midlife and late-life obesity and the risk of dementia: Cardiovascular health study.* Archives of Neurology, 2009. **66**(3): p. 336-342.
7. Hassing, L.B., et al., *Overweight in midlife and risk of dementia: a 40-year follow-up study.* Int J Obes, 2009. **33**(8): p. 893-898.
8. Beydoun, M.A., et al., *Association of Adiposity Status and Changes in Early to Mid-Adulthood With Incidence of Alzheimer's Disease.* American Journal of Epidemiology, 2008. **168**(10): p. 1179-1189.
9. Norton, S., et al., *Potential for primary prevention of Alzheimer's disease: an analysis of population-based data.* The Lancet Neurology, 2014. **13**(8): p. 788-794.
10. Arnoldussen, I.A.C., A.J. Kiliaan, and D.R. Gustafson, *Obesity and dementia: Adipokines interact with the brain.* European Neuropsychopharmacology, 2014. **24**(12): p. 1982-1999.
11. Grant, W.B., *Dietary Links to Alzheimer's Disease: 1999 Update.* Journal of Alzheimer's Disease, 1999. **1**(4): p. 197-201.
12. Grant, W.B., *Dietary Links to Alzheimer's Disease.* **Alzheimer's Disease**, 1997. **Rev. 2**: p. 42-55.
13. Huang, Y. and L. Mucke, *Alzheimer Mechanisms and Therapeutic Strategies.* Cell, 2012. **148**(6): p. 1204-1222.
14. Kanoski, S.E., et al., *The Effects of a High-Energy Diet on Hippocampal Function and Blood-Brain Barrier Integrity in the Rat.* Journal of Alzheimer's Disease, 2010. **21**(1): p. 207-219.
15. Boitard, C., et al., *Impairment of hippocampal-dependent memory induced by juvenile high-fat diet intake is associated with enhanced hippocampal inflammation in rats.* Brain, Behavior, and Immunity, 2014. **40**(0): p. 9-17.
16. Valladolid-Acebes, I., et al., *Spatial memory impairment and changes in hippocampal morphology are triggered by high-fat diets in adolescent mice. Is there a role of leptin?* Neurobiology of Learning and Memory, 2013. **106**(0): p. 18-25.
17. Valladolid-Acebes, I., et al., *High-fat diets impair spatial learning in the radial-arm maze in mice.* Neurobiology of Learning and Memory, 2011. **95**(1): p. 80-85.
18. Heyward, F.D., et al., *Adult mice maintained on a high-fat diet exhibit object location memory deficits and reduced hippocampal SIRT1 gene expression.* Neurobiology of Learning and Memory, 2012. **98**(1): p. 25-32.
19. Reichelt, A.C., et al., *Dietary-induced obesity disrupts trace fear conditioning and decreases hippocampal reelin expression.* Brain, Behavior, and Immunity, 2015. **43**: p. 68-75.
20. Sobesky, J.L., et al., *High-fat diet consumption disrupts memory and primes elevations in hippocampal IL-1β, an effect that can be prevented with dietary reversal or IL-1 receptor antagonism.* Brain, Behavior, and Immunity, 2014. **42**: p. 22-32.
21. Arnold, S.E., et al., *High fat diet produces brain insulin resistance, synaptodendritic abnormalities and altered behavior in mice.* Neurobiology of Disease, 2014. **67**(0): p. 79-87.
22. Erion, J.R., et al., *Obesity Elicits Interleukin 1-Mediated Deficits in Hippocampal Synaptic Plasticity.* The Journal of Neuroscience, 2014. **34**(7): p. 2618-2631.

23. Hsu, T.M. and S.E. Kanoski, *Blood-brain barrier disruption: mechanistic links between Western diet consumption and dementia.* Frontiers in Aging Neuroscience, 2014. **6**: p. 88.
24. Sharma, S., R. Taliyan, and S. Ramagiri, *Histone Deacetylase Inhibitor, Trichostatin A, Improves Learning and Memory in High-Fat Diet-Induced Cognitive Deficits in Mice.* Journal of Molecular Neuroscience, 2014: p. 1-11.
25. 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.* Neurobiology of Aging, 2014. **35**(8): p. 1821-1832.
26. Martin, S.A.L., 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.
27. Barron, A.M., et al., *Sex-Specific Effects of High Fat Diet on Indices of Metabolic Syndrome in 3xTg-AD Mice: Implications for Alzheimer's Disease.* PLoS ONE, 2013. **8**(10): p. e78554.
28. Takalo, M., et al., *High-fat diet increases tau expression in the brain of T2DM and AD mice independently of peripheral metabolic status.* The Journal of Nutritional Biochemistry, 2014. **25**(6): p. 634-641.
29. Vandal, M., et al., *Insulin Reverses the High-Fat Diet–Induced Increase in Brain Aβ and Improves Memory in an Animal Model of Alzheimer Disease.* Diabetes, 2014.
30. Ramos-Rodriguez, J.J., et al., *Prediabetes-induced vascular alterations exacerbate central pathology in APPswe/PS1dE9 mice.* Psychoneuroendocrinology, 2014. **48**(0): p. 123-135.
31. Maesako, M., et al., *Environmental enrichment ameliorated high-fat diet-induced Aβ deposition and memory deficit in APP transgenic mice.* Neurobiology of Aging, 2012. **33**(5): p. 1011.e11-1011.e23.
32. Cucciolla, V., et al., *Resveratrol: From Basic Science to the Clinic.* Cell Cycle, 2007. **6**(20): p. 2495-2510.
33. Athar, M., et al., *Resveratrol: A review of preclinical studies for human cancer prevention.* Toxicology and Applied Pharmacology, 2007. **224**(3): p. 274-283.
34. Das, S. and D.K. Das, *Anti-Inflammatory Responses of Resveratrol.* Inflammation & Allergy - Drug Targets (Formerly ???Current Drug Targets, 2007. **6**(3): p. 168-173.
35. Petrovski, G., N. Gurusamy, and D.K. Das, *Resveratrol in cardiovascular health and disease.* Annals of the New York Academy of Sciences, 2011. **1215**(1): p. 22-33.
36. Wang, J., et al., *Moderate consumption of Cabernet Sauvignon attenuates Aβ neuropathology in a mouse model of Alzheimer's disease.* The FASEB Journal, 2006. **20**(13): p. 2313-2320.
37. Anekonda, T.S., *Resveratrol—A boon for treating Alzheimer's disease?* Brain Research Reviews, 2006. **52**(2): p. 316-326.
38. Frozza, R., et al., *Neuroprotective Effects of Resveratrol Against Aβ Administration in Rats are Improved by Lipid-Core Nanocapsules.* Molecular Neurobiology, 2013. **47**(3): p. 1066-1080.
39. Huang, T.-C., et al., *Resveratrol Protects Rats from Aβ-induced Neurotoxicity by the Reduction of iNOS Expression and Lipid Peroxidation.* PLoS ONE, 2011. **6**(12): p. e29102.
40. Karuppagounder, S.S., et al., *Dietary supplementation with resveratrol reduces plaque pathology in a transgenic model of Alzheimer's disease.* Neurochemistry International, 2009. **54**(2): p. 111-118.
41. Lu, C., et al., *Design, Synthesis, and Evaluation of Multitarget-Directed Resveratrol Derivatives for the Treatment of Alzheimer's Disease.* Journal of medicinal chemistry, 2013. **56**(14): p. 5843-5859.
42. Marambaud, P., H. Zhao, and P. Davies, *Resveratrol Promotes Clearance of Alzheimer's Disease Amyloid-β Peptides.* Journal of Biological Chemistry, 2005. **280**(45): p. 37377-37382.
43. Porquet, D., et al., *Dietary resveratrol prevents Alzheimer's markers and increases life span in SAMP8.* Age, 2013. **35**(5): p. 1851-1865.
44. Rege, S.D., et al., *Neuroprotective effects of Resveratrol in Alzheimer Disease Pathology.* Frontiers in Aging Neuroscience, 2014. **6**.
45. Varamini, B., A.K. Sikalidis, and K.L. Bradford, *Resveratrol increases cerebral glycogen synthase kinase phosphorylation as well as protein levels of drebrin and transthyretin in mice: an exploratory study.* International Journal of Food Sciences and Nutrition, 2014. **65**(1): p. 89-96.

46. Vingtdeux, V., et al., *AMP-activated Protein Kinase Signaling Activation by Resveratrol Modulates Amyloid-β Peptide Metabolism.* The Journal of biological chemistry, 2010. **285**(12): p. 9100-9113.
47. Luchsinger, J.A. and R. Mayeux, *Dietary factors and Alzheimer's disease.* The Lancet Neurology, 2004. **3**(10): p. 579-587.
48. Feng, X., et al., *Resveratrol Inhibits β-Amyloid-Induced Neuronal Apoptosis through Regulation of SIRT1-ROCK1 Signaling Pathway.* PLoS ONE, 2013. **8**(3): p. e59888.
49. Baur, J.A., et al., *Resveratrol improves health and survival of mice on a high-calorie diet.* Nature, 2006. **444**(7117): p. 337-342.
50. Gómez-Zorita, S., et al., *Effects of resveratrol on obesity-related inflammation markers in adipose tissue of genetically obese rats.* Nutrition, 2013. **29**(11–12): p. 1374-1380.
51. Jeon, B.T., et al., *Resveratrol Attenuates Obesity-Associated Peripheral and Central Inflammation and Improves Memory Deficit in Mice Fed a High-Fat Diet.* Diabetes, 2012. **61**(6): p. 1444-1454.
52. Kim, S., et al., *Resveratrol exerts anti-obesity effects via mechanisms involving down-regulation of adipogenic and inflammatory processes in mice.* Biochemical Pharmacology, 2011. **81**(11): p. 1343-1351.
53. Nagao, K., et al., *Effect of dietary resveratrol on the metabolic profile of nutrients in obese OLETF rats.* Lipids in Health and Disease, 2013. **12**(1): p. 8.
54. Wang, B., et al., *Resveratrol prevents suppression of regulatory T-cell production, oxidative stress, and inflammation of mice prone or resistant to high-fat diet–induced obesity.* Nutrition Research, 2013. **33**(11): p. 971-981.
55. Zhang, J., et al., *The protective effect of resveratrol on islet insulin secretion and morphology in mice on a high-fat diet.* Diabetes Research and Clinical Practice, 2012. **97**(3): p. 474-482.
56. Chang, H.-C., et al., *Resveratrol Attenuates High-Fat Diet-Induced Disruption of the Blood–Brain Barrier and Protects Brain Neurons from Apoptotic Insults.* Journal of agricultural and food chemistry, 2014. **62**(15): p. 3466-3475.
57. Abraham, J. and R.W. Johnson, *Consuming a Diet Supplemented with Resveratrol Reduced Infection-Related Neuroinflammation and Deficits in Working Memory in Aged Mice.* Rejuvenation Research, 2009. **12**(6): p. 445-453.
58. Anastácio, J.R., et al., *Resveratrol treatment has neuroprotective effects and prevents cognitive impairment after chronic cerebral hypoperfusion.* Neurological Research, 2014. **36**(7): p. 627-633.
59. Liu, D., et al., *Resveratrol prevents impaired cognition induced by chronic unpredictable mild stress in rats.* Progress in Neuro-Psychopharmacology and Biological Psychiatry, 2014. **49**(0): p. 21-29.
60. Liu, G.-S., et al., *Resveratrol attenuates oxidative damage and ameliorates cognitive impairment in the brain of senescence-accelerated mice.* Life Sciences, 2012. **91**(17–18): p. 872-877.
61. Schmatz, R., et al., *Resveratrol prevents memory deficits and the increase in acetylcholinesterase activity in streptozotocin-induced diabetic rats.* European Journal of Pharmacology, 2009. **610**(1–3): p. 42-48.
62. Yazir, Y., et al., *Resveratrol exerts anti-inflammatory and neuroprotective effects to prevent memory deficits in rats exposed to chronic unpredictable mild stress.* Physiology & Behavior, 2015. **138**(0): p. 297-304.
63. Porquet, D., et al., *Neuroprotective Role of Trans-Resveratrol in a Murine Model of Familial Alzheimer's Disease.* Journal of Alzheimer's Disease, 2014. **42**(4): p. 1209-1220.
64. Barger, J.L., et al., *A Low Dose of Dietary Resveratrol Partially Mimics Caloric Restriction and Retards Aging Parameters in Mice.* PLoS ONE, 2008. **3**(6): p. e2264.
65. Mouton, P.R., et al., *Caloric restriction attenuates amyloid deposition in middle-aged dtg APP/PS1 mice.* Neuroscience Letters, 2009. **464**(3): p. 184-187.
66. Patel, N.V., et al., *Caloric restriction attenuates Aβ-deposition in Alzheimer transgenic models.* Neurobiology of Aging, 2005. **26**(7): p. 995-1000.
67. Wang, J., et al., *Caloric restriction attenuates β-amyloid neuropathology in a mouse model of Alzheimer's disease.* The FASEB Journal, 2005.

68. Halagappa, V.K.M., et al., *Intermittent fasting and caloric restriction ameliorate age-related behavioral deficits in the triple-transgenic mouse model of Alzheimer's disease.* Neurobiology of Disease, 2007. **26**(1): p. 212-220.
69. 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 - 421.
70. Ennaceur, A. and J. Delacour, *A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data.* Behavioural Brain Research, 1988. **31**(1): p. 47-59.
71. Baur, J.A. and D.A. Sinclair, *Therapeutic potential of resveratrol: the in vivo evidence.* Nat Rev Drug Discov, 2006. **5**(6): p. 493-506.
72. Lagouge, M., et al., *Resveratrol Improves Mitochondrial Function and Protects against Metabolic Disease by Activating SIRT1 and PGC-1α.* Cell, 2006. **127**(6): p. 1109-1122.
73. Alberdi, G., et al., *Changes in white adipose tissue metabolism induced by resveratrol in rats.* Nutrition & Metabolism, 2011. **8**: p. 29-29.
74. Chen, Q., et al., *Dietary resveratrol increases the expression of hepatic 7α-hydroxylase and ameliorates hypercholesterolemia in high-fat fed C57BL/6J mice.* Lipids in Health and Disease, 2012. **11**: p. 56-56.
75. Alberdi, G., et al., *Thermogenesis is involved in the body-fat lowering effects of resveratrol in rats.* Food Chemistry, 2013. **141**(2): p. 1530-1535.
76. Poulsen, M.M., et al., *Resveratrol up-regulates hepatic uncoupling protein 2 and prevents development of nonalcoholic fatty liver disease in rats fed a high-fat diet.* Nutrition Research, 2012. **32**(9): p. 701-708.
77. Knight, E.M., et al., *Hypermetabolism in a triple-transgenic mouse model of Alzheimer's disease.* Neurobiology of Aging, 2012. **33**(1): p. 187-193.
78. Saito, M., et al., *High Incidence of Metabolically Active Brown Adipose Tissue in Healthy Adult Humans: Effects of Cold Exposure and Adiposity.* Diabetes, 2009. **58**(7): p. 1526-1531.
79. Cypess, A.M., et al., *Identification and Importance of Brown Adipose Tissue in Adult Humans.* The New England journal of medicine, 2009. **360**(15): p. 1509-1517.
80. Stanford, K.I., et al., *Brown adipose tissue regulates glucose homeostasis and insulin sensitivity.* The Journal of Clinical Investigation, 2013. **123**(1): p. 215-223.
81. Zhang, Y., et al., *Targeted deletion of thioesterase superfamily member 1 promotes energy expenditure and protects against obesity and insulin resistance.* Proceedings of the National Academy of Sciences, 2012. **109**(14): p. 5417-5422.
82. Zhang, Y., et al., *Medium-Chain Triglyceride Activated Brown Adipose Tissue and Induced Reduction of Fat Mass in C57BL/6J Mice Fed High-fat Diet.* Biomed Environ Sci, 2015. **28**(2): p. 97-104.
83. Xu, X., et al., *Exercise ameliorates high-fat diet-induced metabolic and vascular dysfunction, and increases adipocyte progenitor cell population in brown adipose tissue.* American Journal of Physiology - Regulatory, Integrative and Comparative Physiology, 2011. **300**(5): p. R1115-R1125.
84. Cinti, S., *Transdifferentiation properties of adipocytes in the adipose organ*. Vol. 297. 2009. E977-E986.
85. Scarpace, P.J., M. Matheny, and S.E. Borst, *Thermogenesis and mitochondrial GDP binding with age in response to the novel agonist CGP-12177A.* Am J Physiol, 1992. **262**: p. E185–E190.
86. Saely, C.H., K. Geiger, and H. Drexel, *Brown versus White Adipose Tissue: A Mini-Review.* Gerontology, 2012. **58**(1): p. 15-23.
87. Peng, X.l., et al., *Resveratrol Ameliorates High Glucose and High-Fat/Sucrose Diet-Induced Vascular Hyperpermeability Involving Cav-1/eNOS Regulation.* PLoS ONE, 2014. **9**(11): p. e113716.
88. Montero, M., et al., *Effects of Long-Term Feeding of the Polyphenols Resveratrol and Kaempferol in Obese Mice.* PLoS ONE, 2014. **9**(11): p. e112825.
89. Wang, B., et al., *Regulatory effects of resveratrol on glucose metabolism and T-lymphocyte subsets in the development of high-fat diet-induced obesity in C57BL/6 mice.* Food & Function, 2014. **5**(7): p. 1452-1463.

90. Reichelt, A.C., et al., *Dietary-induced obesity disrupts trace fear conditioning and decreases hippocampal reelin expression.* Brain, Behavior, and Immunity, (0).
91. Ouyang, S., et al., *Diet-induced obesity suppresses expression of many proteins at the blood-brain barrier.* J Cereb Blood Flow Metab, 2014. **34**(1): p. 43-51.
92. Davidson, T.L., et al., *The effects of a high-energy diet on hippocampal-dependent discrimination performance and blood–brain barrier integrity differ for diet-induced obese and diet-resistant rats.* Physiology & Behavior, 2012. **107**(1): p. 26-33.
93. Stranahan, A.M., et al., *Diet-induced insulin resistance impairs hippocampal synaptic plasticity and cognition in middle-aged rats.* Hippocampus, 2008. **18**(11): p. 1085-1088.
94. McNeilly, A.D., et al., *High fat feeding promotes simultaneous decline in insulin sensitivity and cognitive performance in a delayed matching and non-matching to position task.* Behavioural Brain Research, 2011. **217**(1): p. 134-141.
95. McNay, E.C., et al., *Hippocampal memory processes are modulated by insulin and high-fat-induced insulin resistance.* Neurobiology of Learning and Memory, 2010. **93**(4): p. 546-553.

Supplemental Material

Attenuation of obese phenotype by resveratrol does not improve memory in high-fat diet fed mice

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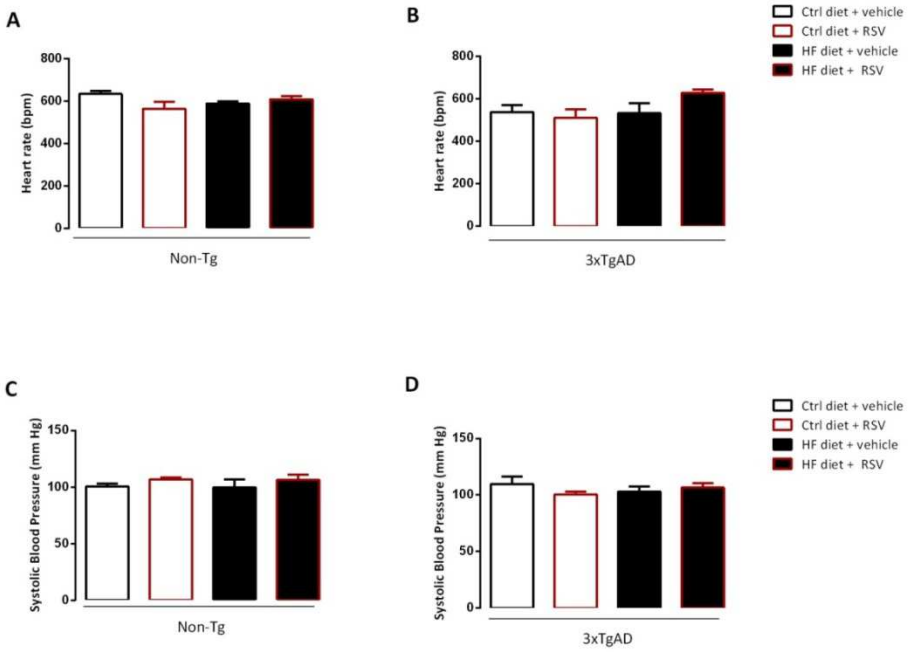
Supplementary Methods

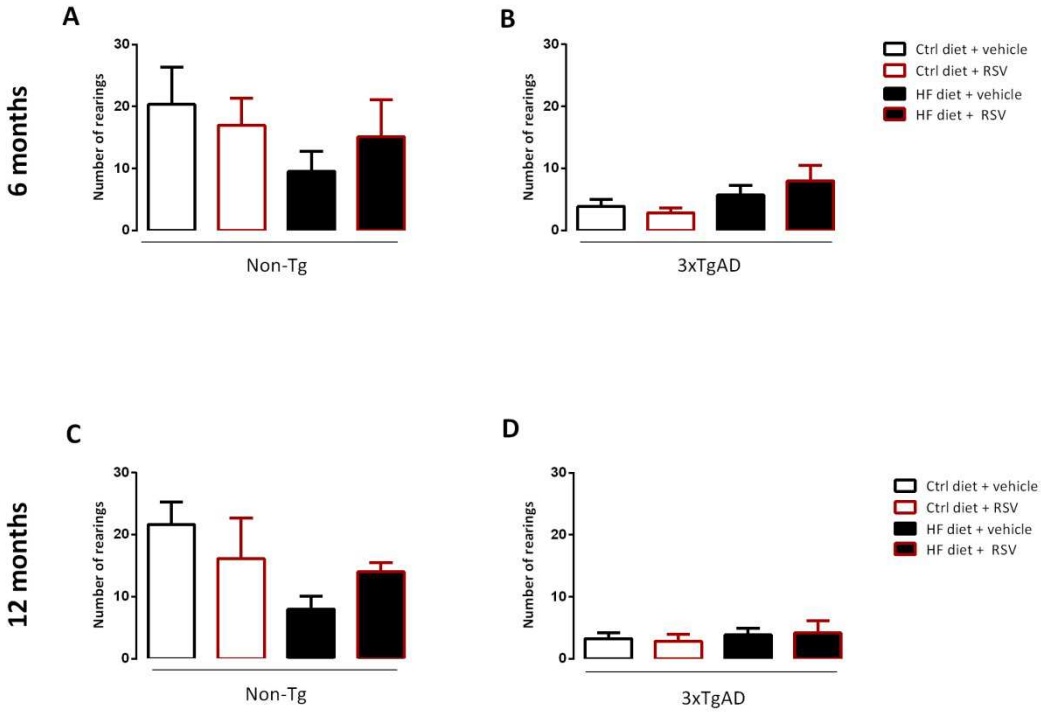
Blood pressure

Systolic blood pressure and heart rate were assessed at 6 months using a non-invasive blood pressure analyser with a specific mouse tail cuff adapter (Model BP-2000-M-2, Visitech Systems, Inc, USA). One day prior to measurements, mice were habituated to the procedure for 5 minutes to reduce stress and anxiety. On the day of the assessment the animals were placed in the room and allowed to acclimatise for 10 minutes. A total of 20 readings of systolic blood pressure (mmHg) and heart rate (beats/minute, bpm) were taken and the average of the last 10 measurements calculated.

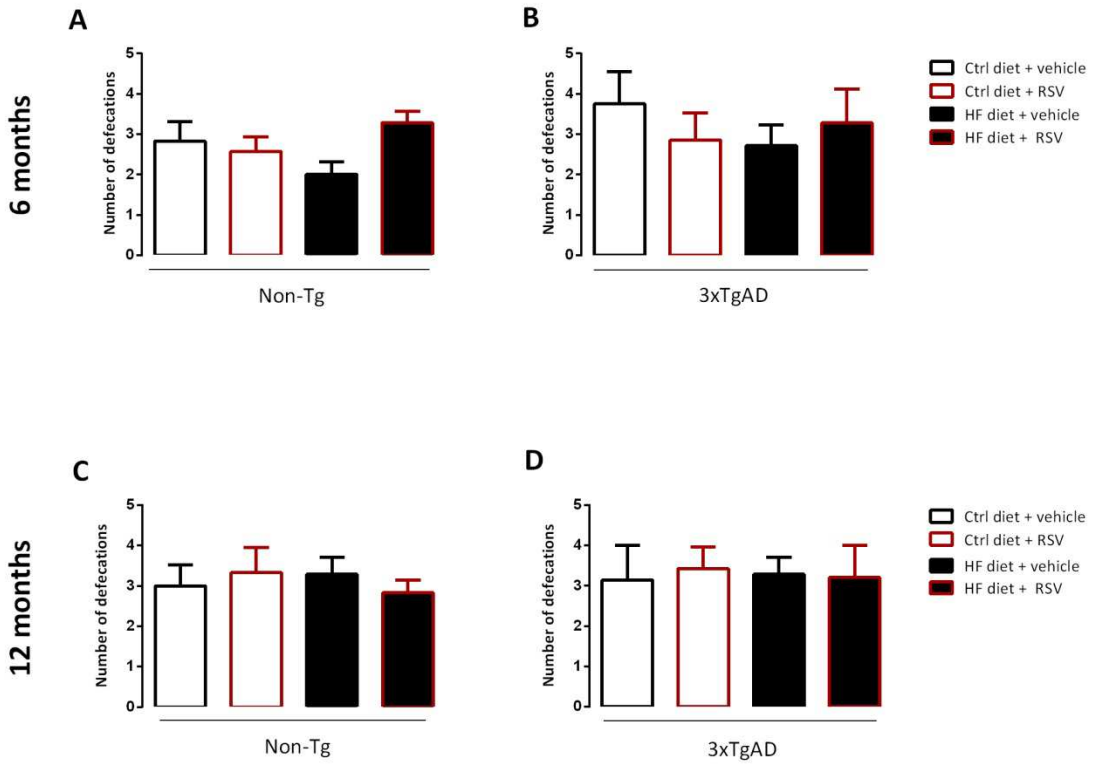
Supplementary results



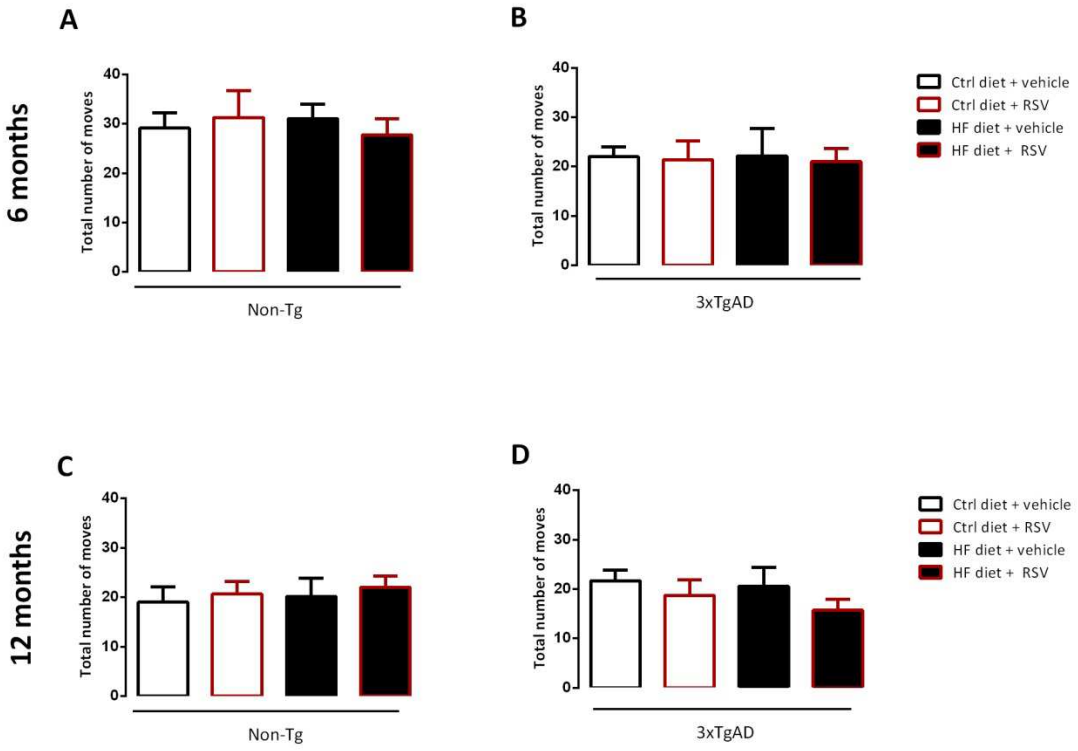
Supplementary Figure 1. Heart rate and systolic blood pressure were unchanged with high-fat diet or RSV treatment after 6 months. Groups of female Non-Tg and 3xTgAD mice were maintained on a high-fat diet and treated with vehicle (HF diet & vehicle) or RSV (HF diet & RSV) at 5 mg/kg body weight/day, or on a control diet and treated with vehicle (Ctrl diet & vehicle) or RSV (Ctrl diet & RSV) from 5 weeks of age. After 6 months heart rate (beats/minute, bpm) and systolic blood pressure (mmHg) were assessed. Data are mean ± SEM, n = 6-7/group.



Supplementary Figure 2. RSV or high-fat diet had no effect in the number of rearings in Non-Tg control or 3xTgAD mice in the open-field test after 6 and 12 months. Groups of female Non-Tg and 3xTgAD mice were maintained on a high-fat diet and treated with vehicle (HF diet & vehicle) or RSV (HF diet & RSV) at 5 mg/kg body weight/day, or on a control diet and treated with vehicle (Ctrl diet & vehicle) or RSV (Ctrl diet & RSV) from 5 weeks of age. The number of rearings was assessed in the open-field test after 6 and 12 months. Data are mean ± SEM, n = 6-7/group.



Supplementary Figure 3. RSV and high-fat diet do not affect the number of defecations in the open-field test in female Non-Tg control and 3xTgAD mice after 6 and 12 months. Groups of female Non-Tg and 3xTgAD mice were maintained on a high-fat diet and treated with vehicle (HF diet & vehicle) or RSV (HF diet & RSV) at 5 mg/kg body weight/day, or on a control diet treated with vehicle (Ctrl diet & vehicle) or RSV (Ctrl diet & RSV) from 5 weeks of age.The number of defecations was assessed in the open-field test after 6 and 12 months. Data are mean ± SEM, n = 6-7/group.



Supplementary Figure 4. RSV and high-fat diet had no effect on the total number of moves in Y-maze spontaneous alternation test in female Non-Tg control and 3xTgAD mice after 6 and 12 months. Groups of female Non-Tg and 3xTgAD mice were maintained on a high-fat diet treated with vehicle (HF diet & vehicle) or RSV (HF diet & RSV) at 5 mg/Kg body weight/day, or on a control diet treated with vehicle (Ctrl diet & vehicle) or RSV (Ctrl diet & RSV) from 5 weeks of age. The total number of moves was assessed in the Y-maze spontaneous alternation test after 6 and 12 months. Data are mean ± SEM, n = 6-7/group.

Chapter 6

General discussion

6.1 Summary of findings

The work in this thesis was carried out with the aim to investigate the role of a high-fat diet on memory in both cognitively normal and cognitively impaired mice and determine the underlying mechanisms. Memory impairment is a common feature of both obesity and AD and is associated with several other molecular events that are involved in both disorders, such as inflammation, T2D, insulin resistance and BBB impairment. Moreover, the particular role of each molecular phenomenon on cognition, as well the molecular mechanisms behind the effect of a high-fat diet on memory remains unclear. In addition, it is known that some risk factors for AD such as sex, can influence AD prevalence and the same is true for obesity. More women develop AD and in obesity the pattern of fat distribution between women and men is different. This suggests that gender may have an important influence in both AD and obesity and therefore could also affect cognition. Thus the aim of this thesis was to further understand the role of gender on the impact of a high-fat diet on memory in both cognitively normal and cognitively impaired mice and try to understand possible molecular mechanisms for its effect. Therefore the main aims were to:

- evaluate the role of a high-fat diet on both neuropathology (Aβ oligomers) and neuroinflammation (microglia) in male 3xTgAD mice;
- compare the effect of a high-fat diet on memory in male and female Non-Tg and 3xTgAD mice;
- evaluate if memory deficits induced by a high-fat diet and in AD are associated with changes in BBB structure, mitochondria and synapses in male and female Non-Tg and 3xTgAD mice;
- evaluate if the detrimental effects of a high-fat diet are dependent on obesity.

To address the first aim, groups of male 3xTgAD mice were maintained on a control or a high-fat diet for different periods of time and memory deficits were observed in high-fat fed Non-Tg and 3xTgAD mice (work performed by Elysse Knight, PhD). The level of Aβ oligomers were measured in 3xTgAD mice and markers of neuroinflammation were assessed in both Non-Tg and 3xTgAD mice.

Data demonstrated that memory deficits induced by a high-fat diet in 3xTgAD mice were independent of Aβ oligomers levels. In addition, there was an earlier activation of microglia in high-fat fed 3xTgAD mice compared to control fed 3xTgAd mice. Moreover, a high-fat diet also increased microglia activation in Non-Tg mice after long-term feeding. Overall, these data suggested that cognitive impairment induced by a high-fat diet is independent of Aβ oligomers in male 3xTgAD mice and might involve the activation of microglia in male Non-Tg mice.

Since a high-fat diet impaired memory in both male Non-Tg and 3xTgAD mice the second aim was to assess if gender could influence cognition and, if so, what determine the molecular mechanism underlying that effect. Both male and female Non-Tg and 3xTgAD mice were kept on a control or a high-fat diet for a short or long-term feeding period and measures of obesity and memory monitored. Data demonstrated that male Non-Tg mice were more susceptible to a diet-induced obesity after a short-term feeding period and exhibited earlier deficits in memory in response to a high-fat diet than females. Memory deficits were associated with increased insulin levels in Non-Tg mice. Interestingly, in this study high-fat diet had no impact on memory or insulin levels in both male and female 3xTgAD mice. Furthermore, certain behavioural tests were more sensitive at detecting memory deficits induced by high-fat diet in Non-Tg mice than others. In conclusion, this study demonstrated that insulin signalling might be an important pathway in the molecular mechanisms underlying the detrimental effect of high-fat diet on memory, effects that are sex dependent.

Several features are common between obesity and AD including oxidative stress, mitochondrial dysfunction and BBB impairment, and both can cause memory deficits. As the effect of a high-fat diet on memory appeared to be influenced by sex the third aim was to evaluate if sex could affect the ultrastructure of the BBB and neurones in response to a high-fat diet. Male and female Non-Tg and 3xTgAD mice were fed a control or a high-fat diet for 6 months. Data from the previous study (Chapter 3) shows that at 6 months a high-fat diet causes memory deficits in both male and female Non-Tg mice. It was hypothesised that male Non-Tg mice, due to earlier memory deficits induced by

a high-fat diet and earlier hyperinsulinemia, would present with greater ultrastructural changes in the cerebrovasculature, mitochondria and synapses. Overall, results showed that surprisingly female Non-Tg mice were more sensitive to changes induced by a high-fat diet than males. These changes induced by a high-fat diet in female Non-Tg mice were similar to changes found in control fed female 3xTgAD mice. Specifically, a high-fat diet induced morphological changes in mitochondria and reduced the number of synapses, and this effect was specific to the hippocampus in both Non-Tg and control fed 3xTgAD female mice. However, in 3xTgAD mice a high-fat diet did not further exacerbate these changes. Overall therefore a high-fat diet induced ultrastructural changes in female Non-Tg mice similar to control fed 3xTgAD suggesting that the mechanisms involved in the appearance of these changes might be independent of Aβ and tau pathways, the pathological features of AD.

The final aim was to evaluate if by attenuating the obese phenotype by RSV, a powerful anti-obesogenic compound, the memory impairments could be improved. Female Non-Tg and 3xTgAD mice were maintained on a control or high-fat diet for 12 months with or without RSV treatment. Results demonstrated that RSV attenuated high-fat diet induced obesity in Non-Tg but not in 3xTgAD mice. RSV improved memory performance in control fed 3xTgAD mice but had no effect on memory deficits induced by a high-fat diet in Non-Tg mice. As shown previously a high-fat diet had no effect on memory deficits observed in 3xTgAD mice. These data suggest that the detrimental effect of a high-fat diet on memory is independent of obese phenotype and that its mechanism is likely to be different to those causing cognitive impairment in AD.

Overall the work on this thesis suggests that the effect of a high-fat diet on memory is independent of AD-related mechanisms (Aβ or tau neuropathology) or obese phenotype and appears to be associated with hyperinsulinemia. Moreover, memory deficits induced by a high-fat diet are influenced by sex as male mice show earlier deficits than female mice after short-term feeding, but that over time female mice become more susceptible to changes induced by high-fat diet on mitochondria morphology and synapse number.

6.2 Assessing memory: the importance of behavioural tests

Memory deficits induced by high-fat diet in Non-Tg mice

In this thesis memory deficits were evaluated using a battery of behavioural tests. Data demonstrated that while in some behaviour tests the effect of a high-fat diet on memory was detected rapidly, in other tests the effect was apparent only at a later time during the feeding period, suggesting that different behavioural tests show varying sensitivities in detecting the effects of the high-fat diet. Although there was some lack of consistency between studies reported here as to when memory deficits in response to a high-fat diet were detected (e.g. high-fat feeding induced memory deficits in the Y-maze spontaneous alternation test in female Non-Tg mice at 12 months in Chapter 3 but not in Chapter 5), it is noticeable that deficits in recognition memory (assessed by the novel object recognition test) occur earlier than those for spatial memory (Y-maze spontaneous alternation test). Several studies have demonstrated that a high-fat diet induces memory deficits in cognitively normal mice in both recognition and spatial memory (Pistell et al., 2010, Valladolid-Acebes et al., 2011, Boitard et al., 2012, Heyward et al., 2012, Kaczmarczyk et al., 2013, Arnold et al., 2014, Carey et al., 2014, Camer et al., 2015). In one study using the novel object recognition and Y-maze spontaneous alternation tests to assess memory impairment after a high-fat diet (60% energy from fat), short-term feeding for 1 week impaired memory in both tests, however after 3 weeks of high-fat feeding deficits were only observed in novel object recognition test suggesting that although both tests assess hippocampal-dependent memory they have different sensitivities for detecting high-fat diet induced deficits. The hippocampus is important for both spatial and recognition memory, however how much hippocampal input is needed to support the behavioural performance in each test may differ (Broadbent et al., 2004). Is known that larger hippocampal lesions are needed to impair recognition memory than are required to impair spatial memory (Broadbent et al., 2004). Indeed, in the present study the novel object recognition test is completely dependent on the hippocampus (1 h delay), while the Y-maze spontaneous alternation test, although dependent on the hippocampus, also uses other brain areas such as prefrontal cortex,

basal forebrain and septum (Lalonde, 2002), which suggests that mice can cope better with hippocampal-impairment and can rely on other areas to perform the test. These facts may explain why in the present thesis mice with deficits in the novel object recognition test are still able to successfully perform the Y-maze test. In conclusion, in the present thesis the novel object recognition test is more sensitive test to detect early changes in memory induced by a high-fat diet than the Y-maze spontaneous alternation test.

Whether a memory deficit was detected after a high-fat diet in Non-Tg mice was also different between the novel object recognition and smell recognition tests. Data from the smell recognition test demonstrated that a high-fat diet did not impair memory at any time assessed in Non-Tg mice but an impairment was observed as early as 7 weeks in the novel object recognition test for male mice and 6 months for females (Chapter 3 and 5). This discrepancy might be due to the fact that the sense of smell in mice is vital for survival and how they find food and shelter, so olfactory stimuli may be stronger than visual. Another difference between the smell and novel object recognition tests is the time of the delay, which is only 3 min versus 1 h respectively. This short delay time between the two phases in the smell recognition test could rely more on short-term memory mechanisms, closing related with working memory, than long-term memory, suggesting that perhaps short-term memory is not as affected by a high-fat diet as long-term memory.

As mentioned previously, there is a lack of consistency in the appearance of the memory deficits induced by a high-fat diet in female Non-Tg mice. In Chapter 3 female Non-Tg mice fed with a high-fat diet showed memory impairments; deficits in novel object recognition test at 6 months and Y-maze deficits at 12 months on diet. However, in Chapter 5 female Non-Tg mice did not show impairment in Y-maze spontaneous alternation at 12 months. The main difference between studies is the time that the high-fat diet started: Chapter 3 at 8-weeks-old (young adult); Chapter 5 at 5-weeks-old (adolescent). It has been demonstrated that the time that mice start to consume the high-fat diet (adolescence versus young) is important to the development of the memory deficits, with consumption during adolescence resulting in more memory deficits than during young adulthood (Valladolid-Acebes et al., 2013). Although it is the opposite of what is demonstrated here these data demonstrate that the age can influence the effect of a high-fat diet on memory. In addition, phases

of the ovarian cycles in female mice are with no doubt a key factor that could explain the major differences between female cohorts and should be addressed in future studies.

Memory deficits in 3xTgAD mice

Up to 12 months a high-fat feeding had no effect on memory in both male and female 3xTgAD mice as demonstrated previously by our lab and others (Barron et al., 2013, Knight et al., 2014, Vandal et al., 2014). As memory deficits were apparent in control-fed 3xTgAD mice at all time points when assessed by the novel object and smell recognition tests, a further effect of a high-fat diet could not be assessed here, although a high-fat diet had no effect on memory in 3xTgAD mice when assessed using the Y-maze test up to 12 months. However, as a high-fat diet did not affect memory in 3xTgAD mice in the Y-maze (Chapter 1) until 15-16 months, a longer period of feeding might be needed to see an effect in the present study. Moreover, in both male and female 3xTgAD mice a high-fat diet did not induce hyperinsulinemia, which could explain the lack of memory deficits in Y-maze spontaneous alternation.

In both male and female control-fed 3xTgAD mice, early hippocampal-dependent deficits in memory were seen when assessed by the novel and smell object recognition tests. In the Y-maze spontaneous alternation test, control-fed female 3xTgAD mice had memory deficits whereas males did not. This result is supported by others who demonstrated that female 3xTgAD mice have greater memory impairments than male 3xTgAD mice (Clinton et al., 2007, Blázquez et al., 2014) which could be due to females exhibiting more extensive amyloid pathology (Hirata-Fukae et al., 2008).

6.3 Insulin, mitochondria and synapses: are they involved in the effects of a high-fat diet on memory?

The role of insulin in memory impairment induced by a high-fat diet

The research presented in this thesis show that male Non-Tg mice are more susceptible to memory deficits induced by a high-fat diet than females, as deficits were detected earlier. This is consistent with a study by Hwang and colleagues showing that male mice are more vulnerable to high-fat diet induced learning impairments and synaptic plasticity (Hwang et al., 2010), however these results were obtained during long-term feeding (9-12 months). In the present thesis results suggest that earlier memory impairments in male high-fat fed Non-Tg mice might be due to a greater increase in body weight gain. Although both male and female Non-Tg mice gained weight after a high-fat diet, an earlier increase in fat content and insulin were observed in males. Interestingly, Hwang and colleagues also observe that deficits in learning and synaptic plasticity are associated with more severe obesity and metabolic dysfunction in male mice than in females, as male mice present with hyperglycemia, more severe hyperinsulinemia and have a greater increased in body weight than females (Hwang et al., 2010).

In rodents fed with a high-fat diet, raised peripheral insulin levels are associated with increased markers of insulin resistance (Arnold et al., 2014), decreased levels of brain insulin within the hippocampus (Sharma and Taliyan, 2014), decreased levels of hippocampal PSD-95 (Arnold et al., 2014), increased levels of oxidative stress (Sharma and Taliyan, 2014) and impairment of cognitive function (Stranahan et al., 2008, McNay et al., 2010, McNeilly et al., 2011, Arnold et al., 2014, Sharma and Taliyan, 2014). High levels of peripheral insulin, associated with insulin resistance, are seen in T2D and obese patients and are accompanied by lower levels of insulin and impaired insulin signalling in the brain (Stockhorst et al., 2004). This shift in the proportion of insulin levels in the brain compared to the periphery in obesity may result from a reduced transport of insulin at the BBB (Stockhorst et al., 2004). Chronic exposure to high levels of insulin will saturate insulin receptors leading to a decrease uptake of insulin into the brain. Since insulin receptors are widely expressed

throughout the brain, in both neurones and glial cells, particularly in the hippocampus (Son et al., 2011), a reduction in insulin levels will therefore have detrimental effects on cognition. Moreover, it has been shown that in the hippocampus insulin receptors respond to learning experiences by altering gene expression and activating downstream molecules that are involved in an early stage of memory formation (Zhao et al., 1999). Taken together these findings suggest that due to earlier increases in peripheral insulin levels after a high-fat diet, male Non-Tg mice might have less insulin uptake into the brain resulting in less efficient memory mechanisms. After long-term high-fat feeding both male and female Non-Tg had raised plasma insulin levels that might also result in reduced brain levels and that could play a role in the memory deficits induced by a high-fat diet at this time.

In addition to the role of insulin in obesity, it is known that insulin resistance also occurs in AD patients and it is correlated with the memory impairments (Craft et al., 1998, Frölich et al., 1998, Craft, 2005, Ghasemi et al., 2013, De Felice and Ferreira, 2014). In 3xTgAD mice, a high-fat diet reduces insulin sensitivity and increases amyloid brain pathology (Barron et al., 2013, Vandal et al., 2014). In addition, a single insulin injection can reverse the effects of a high-fat diet on memory impairment in 3xTgAD mice and reduce soluble Aβ levels (Vandal et al., 2014). This effect of insulin administration has been also observed through intranasal insulin administration in 3xTgAD mice that also restored brain insulin signalling (Chen et al., 2014). Intranasal administration of insulin also has beneficial effects on cognition in animal studies and in clinical trials (Benedict et al., 2007, Craft et al., 2012, Hölscher, 2014, Claxton et al., 2015). These findings suggest that insulin signalling might be the mutual pathway between obesity and AD, and it might have an important role in memory impairment in both conditions.

The role of BBB, mitochondria and synapses on memory deficits induced by a high-fat diet

Since high-fat diet induced impairments in memory were detected earlier in male compared to female Non-Tg mice, it was hypothesised that male Non-Tg mice at 6 months would be more susceptible to ultrastructural changes induced by a high-fat diet in the BBB, mitochondria and synapses. However, results demonstrated that in response to a high-fat diet female Non-Tg mice

showed greater changes in the brain, including elongated endothelial mitochondria, reduced number of neuronal mitochondria and reduced number of synapses, changes that were specific to hippocampus. Moreover, these same changes, plus elongated neuronal mitochondria, were also observed in female control fed 3xTgAD mice. These results suggest that the morphological alterations induced by high-fat diet in Non-Tg mice might share similar mechanisms to those induced by AD. The reason why female Non-Tg mice appear to be more vulnerable to ultrastructural changes induced by a high-fat diet than males, when males present earlier memory deficits, remains to be understood.

Dysfunctional mitochondria, due to increase levels of ROS or impaired mitochondrial redox homeostasis, can affect neuronal function (De Felice and Ferreira, 2014), although it is known that transient production of ROS is implicated in synaptic signalling and facilitates hippocampal LTP and memory mechanisms (Serrano and Klann, 2004, De Felice and Ferreira, 2014). However, when there is a chronic production of ROS and increases in oxidative stress this can impair the mechanisms underlying memory. High-fat diets can increase the production of mitochondrial ROS in skeletal muscle which is thought to be involved in peripheral insulin resistance. This increase in peripheral ROS also increases the level of oxidative stress products in the plasma, such as lipid peroxidation products, that through the bloodstream can reach the brain (De Felice and Ferreira, 2014). In AD it is known that brain insulin signalling and oxidative stress are intimately related, and that mitochondrial dysfunction and increased levels of ROS sustain a vicious cycle that impairs insulin signalling and leads to brain insulin resistance (De Felice and Ferreira, 2014). Insulin has been shown to be involved in mitochondrial function and in the dynamic process of fusion and fission (del Campo et al., 2014, Parra et al., 2014, Santos et al., 2014). Finally, as neurones are critically sensitive to alterations in mitochondrial dynamics and function, due to their dependency on mitochondria for energy production (Correia et al., 2012), is not surprising that a reduction in mitochondrial number and an imbalance of mitochondrial dynamics was associated with a decrease in synaptic number. In summary therefore, it is likely that the mechanisms underlying the effects of a high-fat diet on memory in Non-Tg mice are linked to mitochondrial dysfunction and oxidative stress that could result from central insulin resistance due to peripheral hyperinsulinemia.

6.4 Obesity phenotype is not involved in the effect of a high-fat diet on memory

Obesity is associated with cognitive decline. However is still not clear what mechanisms are behind the detrimental effects of a high-fat diet on memory and if obesity phenotype is involved. In the present thesis RSV, a known anti-obesogenic agent, was used to treat female Non-Tg and 3xTgAD in order to potentially reverse the obese phenotype and test its effect on memory impairment induced by a high-fat diet. The hypothesis was that by reducing body weight gain and fat accumulation the impairment in memory after a high-fat diet would be improved, as it is known that the amount of adiposity and body weight are correlated with cognitive performance in humans (Elias et al., 2003, Wolf et al., 2007). Data demonstrated that RSV was able to reverse the body weight gain and fat accumulation induced by a high-fat diet in female Non-Tg mice but it did not affect the memory deficits. On the other hand, in 3xTgAD mice fed with a high-fat diet RSV had no effect on body weight or WAT accumulation but in control fed mice RSV was able to improve cognition in the smell recognition test. These data give further support that the mechanism associated with memory deficits in high-fat fed Non-Tg mice are likely to be different to those causing memory impairment in AD and that 3xTgAD mice are metabolically different from Non-Tg mice.

As the negative effects of a high-fat diet on memory were still present even when obesity was reduced this suggests that other mechanisms independent of the obese phenotype are involved. As hypothesised, a decrease in fat content would be preventive as less pro-inflammatory cytokines would be released from WAT. However, data from the RSV study demonstrated that reducing WAT content was not sufficient to reverse the effects of a high-fat diet on memory. This data suggests that the diet content had a stronger effect than the extent of adipose tissue.

Consumption of a high-fat diet will increase SFAs levels in the circulation, which could have potential detrimental effects on the hippocampus, such as increase neuroinflammation (e.g activation of microglia) (Molteni et al., 2002, Pistell et al., 2010, Knight et al., 2014). Moreover, it is known that SFAs can acutely cause insulin resistance independent of adiposity (Estadella et al.,

2013), suggesting that insulin resistance might be the cause for the memory impairments observed, however as insulin was not measured in this study this would need to be addressed in future.

6.5 Potential mechanisms for memory deficits associated with a high-fat diet: a link to AD

From the data in the present thesis it is possible to speculate a potential mechanism for the effects of a high-fat diet on memory in Non-Tg mice and also for memory impairments in 3xTgAD mice. Consumption of a high-fat diet increases plasma SFAs and subsequent expansion of WAT. SFAs can increase the production of cytokines, such as TNF-α, from hypertrophic adipocytes and macrophages, resulting in a deterioration of insulin sensitivity (Estadella et al., 2013) (Figure 5). TNF-α increases in the serum of high-fat fed mice (Jeon et al., 2012). Increased levels of TNF-α will lead to serine phosphorylation of IRS-1, which will disrupt insulin signalling (Balistreri et al., 2010). A reduction in insulin signalling will lead to an increase in plasma insulin levels from the pancreas, leading to hyperinsulinemia (Shanik et al., 2008), Over time, peripheral tissues will become insulin resistant. Peripheral insulin resistance will down-regulate insulin uptake at the BBB which will result in decreased levels of insulin in the brain. Reduced transport of insulin into the brain and insulin receptors activity will compromise brain glucose metabolism and insulin signalling pathways (Correia et al., 2012). Abnormalities in brain insulin metabolism can increase oxidative stress that can impair mitochondria function. Mitochondrial dysfunction can influence mitochondrial biogenesis and transport, which can result in a decrease in mitochondrial number (Figure 5). In particular in the hippocampus, where insulin has proven to be vital for processes of memory and learning, mitochondrial integrity is essential for normal neuronal activity. Therefore, damaged mitochondria will affect synapses and ultimately lead to their degeneration, which will affect cognitive performance (Figure 5). Over time (> 12 months), all these events will cause neuroinflammation and an increase in microglia activation (Figure 5). Alternatively, it is known that SFAs alone can also acutely cause peripheral insulin resistance and impair insulin signalling independently of adipose tissue (Estadella et al., 2013), promoting the downstream events outlined above (Figure 5). In

addition, SFAs can affect hippocampal integrity by increasing neuroinflammation (microglia) (Pistell et al., 2010), an effect that are associated with cognitive impairment in mice, although the exact mechanisms remains unclear. These data could explain why female high-fat fed Non-Tg mice still had memory deficits even though RSV reduced adipose tissue and body weight. Finally inflammation of adipose tissue can also induce oxidative stress and promote ROS production (Miller and Spencer, 2014). Moreover, long-term feeding can induce peripheral oxidative stress, characterised by increases in plasma ROS and lipid peroxidation products (Oliveros et al., 2004). Increases in plasma ROS can act on the BBB and induce increased endothelial oxidative stress, which can affect mitochondria dynamics (Figure 5).

In 3xTgAD mice the increase production of Aβ and Aβ deposition, as a result of the progression of the disease, might be the cause for the memory impairments. Brain insulin resistance is known to occur in AD, and insulin resistance has been proposed to be a key in neuropathological events, including Aβ deposition and tau phosphorylation (Correia et al., 2012). A clear relationship between insulin and Aβ metabolism has been documented (Gasparini et al., 2002, Phiel et al., 2003). Aβ oligomers are known to disrupt insulin signalling (Zhao et al., 2008) and IDE activity is decreased in AD, leading to insufficient degradation of Aβ and subsequent Aβ accumulation (Correia et al., 2012). In addition, decreased levels of insulin and impairment of insulin signalling are known to affect mitochondria function and increase oxidative stress. Abnormal Aβ deposition also promotes mitochondrial dysfunction and exacerbates oxidative stress that according to the mitochondrial cascade hypothesis is also a main trigger for cognitive decline, ultimately resulting in memory impairment (Figure 6).

Overall therefore, these potential mechanisms described above based on mitochondrial and insulin impairments might explain how obesity, and in particular high-fat diet consumption, can lead to memory impairments, features that are also observed in AD.

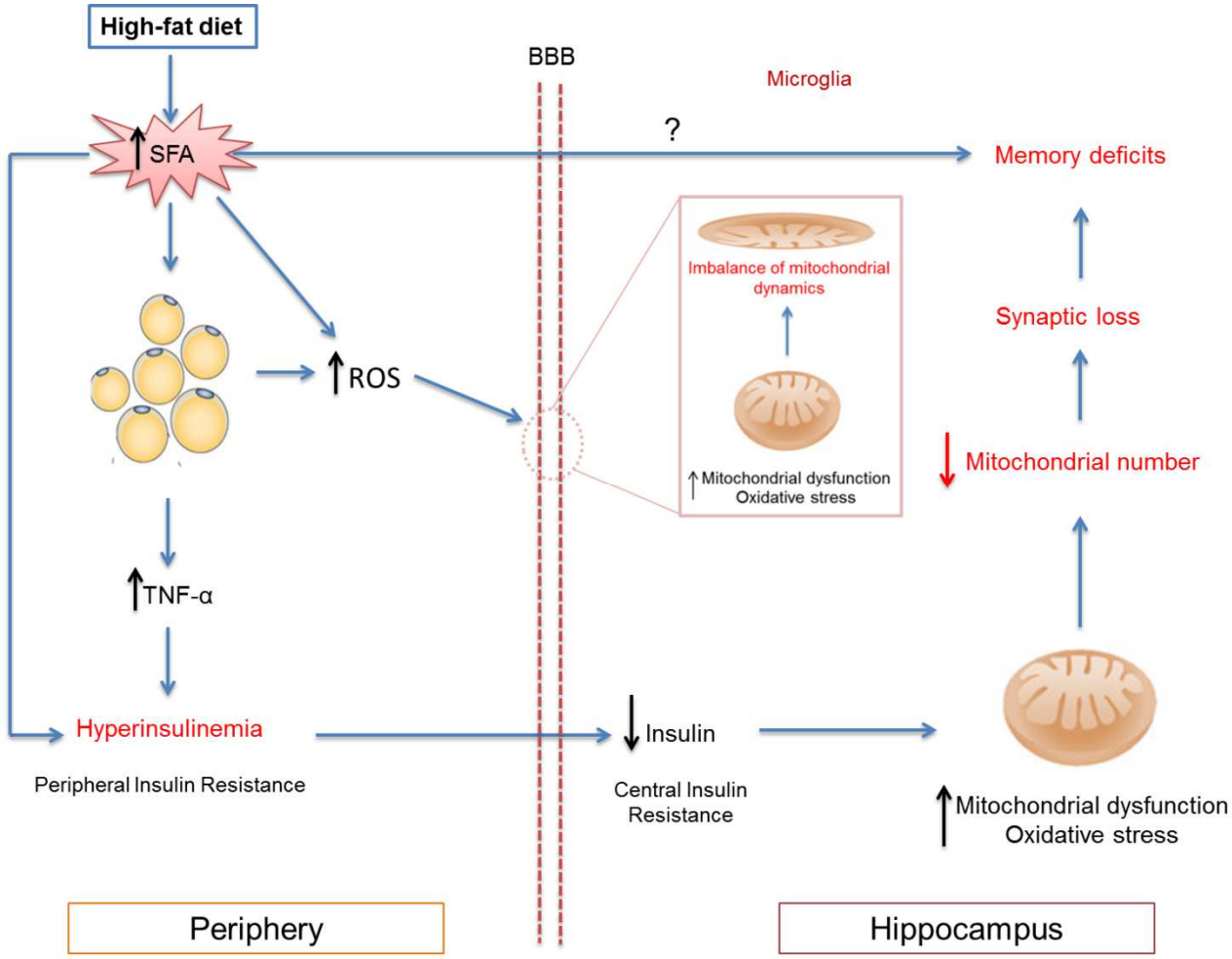


Figure 5. Potential mechanism of memory deficits induced by a high-fat diet. High-fat diet induces memory deficits in Non-Tg mice through peripheral and central insulin resistance. The increase levels of saturated fatty acids (SFA) from the diet cause inflammation of the adipose tissue that produces increased levels of the tumor necrosis factor α (TNF-α) causing a rise in the peripheral levels of insulin (hyperinsulinemia). Increased levels of peripheral insulin then cause down-regulation of insulin uptake into the brain, resulting in central insulin resistance. Decrease brain insulin levels and impairment of the insulin signalling cause oxidative stress and mitochondrial dysfunction ultimately leading to synaptic loss and cognitive impairment. Changes in mitochondria are also observed in the endothelial cells of the blood-brain barrier (BBB) as a result of the increase peripheral reactive species of oxygen (ROS) production induced by high-fat diet. Over time there is an increase of neuroinflammation characterised by increased microglia activation. SFA might also have a direct effect on memory.

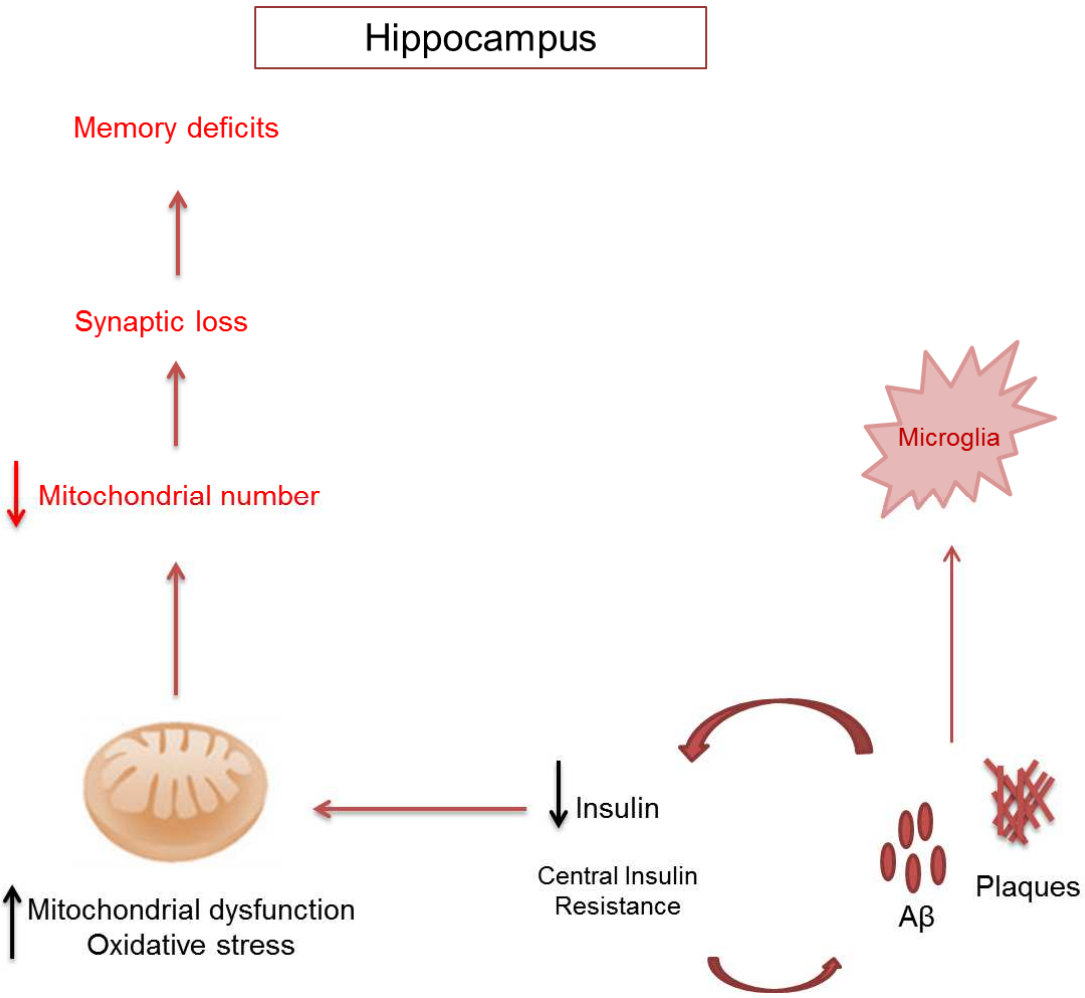


Figure 6. Memory impairment in 3xTgAD mice. In 3xTgAD mice memory impairments are induced by increased amyloid-beta deposits (Aβ) and by insulin signalling impairment, which causes increased oxidative stress and mitochondrial dysfunction. Increased neuroinflammation and activated microglia are also observed. Mitochondrial dysfunction will then lead to synaptic loss and cognitive deficits.

6.6 Future directions

Brain insulin signalling pathways and mitochondrial proteins

In the present thesis only plasma insulin levels were assessed. In order to better understand the role of insulin and its mechanisms underlying memory impairment induced by a high-fat diet both central and peripheral insulin signalling should be assessed. In periphery, adipose tissue is known to be a key factor involved in the level of insulin resistance, while in the brain impairment of insulin is detrimental for memory processes. However, exactly what pathways are involved and whether there is a complete impairment of insulin signalling or just some parts of the cascade are important to determine in order to gain a better understanding of the effects of a high-fat diet. In addition, it would be interesting to understand how insulin pathways influence proteins involved in the dynamic processes in mitochondria and what mitochondrial proteins are affected during insulin resistance.

Can memory deficits be reversed with insulin?

Reducing body weight gain and adipose tissue did not prove to be successful in reversing the detrimental effects of a high-fat diet on memory in Non-Tg mice. Several studies have demonstrated that intranasal administration of insulin can promote cognition. Therefore future studies could assess if intranasal insulin can reverse the memory deficits induced by high-fat diet. Moreover, the effect of insulin administration on mitochondrial morphology and synapses number should also be tested.

Role of peripheral and central inflammation

In Non-Tg mice, microglia activation occurred only after long-term high-fat feeding (15 months), however, other markers of inflammation are known to increase during high-fat feeding. What is the exact role of neuroinflammation and why does appear to occur so late during a high-fat diet? Is there an exacerbation of peripheral inflammation in response to a high-fat diet? Can peripheral inflammation be involved in memory impairment after a high-fat diet and how?

Is there a role for Aβ in Non-Tg mice?

Some studies report that adipose tissue can produce Aβ and that a high-fat diet can promote its production. Moreover, high-fat diets can change gut microbiota, which can lead to production of enterocytic Aβ (Hsu and Kanoski, 2014). Does peripheral Aβ play a role in a factor in why obesity can increase AD prevalence?

6.7 Conclusion

The present thesis aimed to understand the effect of sex on the detrimental effect of a high-fat diet on memory in cognitively normal (Non-Tg mice) and cognitively impaired mice (an AD mouse model, 3xTgAD mice). Data demonstrated that a high-fat diet impaired memory in both male and female Non-Tg mice but had no effect in 3xTgAD mice over the time studied. Although male Non-Tg mice were more vulnerable to short-term high-fat feeding than females by presenting earlier deficits in memory and hyperinsulinemia, after longer periods of high-fat feeding female Non-Tg mice appeared more susceptible, as ultrastructural alterations including changes in mitochondria morphology and number and decreased synaptic number in hippocampus were observed in female mice only. Overall in Non-Tg mice, hippocampal-dependent memory deficits in response to a high-fat diet appeared to be associated with hyperinsulinemia, mitochondrial morphological abnormalities and synaptic loss, but not with obesity and increased adiposity. These data suggest that mechanisms underlying the detrimental effects of a high-fat diet on memory are sex-dependent and could potentially depend on insulin signalling and mitochondrial dysfunction. Therefore, the work in this thesis has increased our understanding about the molecular mechanisms behind the link between obesity and AD and how they affect memory, and that gender differences should be considered in the design of clinical trials for both diseases.

Chapter 7

Appendix

Composition of Diets

Control diet

Basal Purified Diet w/12% Energy From Fat, Yellow — 58G7

DESCRIPTION

Purified Laboratory Rodent Diet. Modification of TestDiet® 5755 with 12% energy from fat, dyed yellow.

Storage conditions are particularly critical to TestDiet® products, due to the absence of antioxidants or preservative agents. To provide maximum protection against possible changes during storage, store in a dry, cool location. Storage under refrigeration (2° C) is recommended. If long term studies are involved, store the diet at -20° C or colder. Be certain to keep in air tight containers.

*Product Forms Available**	*Catalog #*
Meal	1810736
Meal, Irradiated	1810737
1/2" Pellet	58145
Meal	58168

**Other Forms Available By Request*

INGREDIENTS

Dextrin	48.6172
Casein - Vitamin Free	19.8700
Sucrose	16.5580
Corn Oil	4.7310
RP Mineral Mix #10 (adds 1.29% fiber)	4.7310
RP Vitamin Mix (adds 1.94% sucrose)	1.8924
Inulin	1.4193
Powdered Cellulose	1.4193
Lard	0.4257
Choline Chloride	0.1892
DL-Methionine	0.1419
Yellow Dye	0.0050

Part of the TestDiet® DIO Series

Basal Purified Diet with 45% Energy From Fat
1/2" Pellet - Catalog # 58146 (58G8)
Meal - Catalog # 58169 (58G8)

Basal Purified Diet with 60% Energy From Fat
1/2" Pellet - Catalog # 58167 (58G9)
1/2" Pellet, Irradiated - Catalog # 58170 (58G9)
Meal - Catalog # 1810740 (58G9)
Meal, Irradiated - Catalog # 1810741 (58G9)

Basal Purified Diet with 70% Energy From Fat
Meal - Catalog # 58171 (58H0)

FEEDING DIRECTIONS

Feed ad libitum. Plenty of fresh, clean water should be available at all times.

CAUTION:
Perishable, refrigerate upon receipt.

For experimental use only.

6/28/2005

NUTRITIONAL PROFILE [1]

Protein, %	***18.0***
Arginine, %	0.69
Histidine, %	0.51
Isoleucine, %	0.95
Leucine, %	1.72
Lysine, %	1.44
Methionine, %	0.65
Cystine, %	0.07
Phenylalanine, %	0.95
Tyrosine, %	1.01
Threonine, %	0.77
Tryptophan, %	0.22
Valine, %	1.13
Alanine, %	0.55
Aspartic Acid, %	1.28
Glutamic Acid, %	4.06
Glycine, %	0.38
Proline, %	2.34
Serine, %	1.10
Taurine, %	0.00
Fat, %	***5.2***
Cholesterol, ppm	4
Linoleic Acid, %	2.75
Linolenic Acid, %	0.04
Arachidonic Acid, %	0.00
Omega-3 Fatty Acids, %	0.04
Total Saturated Fatty Acids, %	0.78
Total Monounsaturated Fatty Acids, %	1.32
Fiber (max), %	***4.1***
Carbohydrates, %	***67.0***
Energy (kcal/g) [2]	***3.87***

From:	kcal	%
Protein	0.720	18.6
Fat (ether extract)	0.464	12.0
Carbohydrates	2.680	69.3

Minerals	
Calcium, %	0.57
Phosphorus, %	0.54
Phosphorus (available), %	0.54
Potassium, %	0.38
Magnesium, %	0.07
Sodium, %	0.20
Chlorine, %	0.23
Fluorine, ppm	4.7
Iron, ppm	59
Zinc, ppm	26
Manganese, ppm	62
Copper, ppm	14.2
Cobalt, ppm	3.0
Iodine, ppm	0.54
Chromium, ppm	2.9
Molybdenum, ppm	0.77
Selenium, ppm	0.22
Vitamins	
Vitamin A, IU/g	20.9
Vitamin D-3 (added), IU/g	2.1
Vitamin E, IU/kg	47.4
Vitamin K (as menadione), ppm	9.84
Thiamin Hydrochloride, ppm	19.5
Riboflavin, ppm	18.9
Niacin, ppm	85
Pantothenic Acid, ppm	52
Folic Acid, ppm	3.8
Pyridoxine, ppm	15.6
Biotin, ppm	0.4
Vitamin B-12, mcg/kg	19
Choline Chloride, ppm	1,324
Ascorbic Acid, ppm	0.0

1. Based on the latest ingredient analysis information. Since nutrient composition of natural ingredients varies, analysis will differ accordingly. Nutrients expressed as percent of ration on an As Fed basis except where otherwise indicated.
2. Energy (kcal/gm) - Sum of decimal fractions of protein, fat and carbohydrate x 4,9,4 kcal/gm respectively.

TestDiet
www.testdiet.com

High-fat diet

Basal Purified Diet w/60% Energy From Fat, Blue — 58G9

DESCRIPTION

Basal Purified Diet with 60% Energy From Fat, Dyed Blue is based on TestDiet® Basal Diet 5755.

Storage conditions are particularly critical to TestDiet® products, due to the absence of antioxidants or preservative agents. To provide maximum protection against possible changes during storage, store in a dry, cool location. Storage under refrigeratation (2 ° C) is required. If long term studies are involved, store the diet at -20 ° C or colder. Be certain to keep in air tight containers.

*Product Forms Available**	*Catalog #*
Meal	1810740
Meal, Irradiated	1810741
1/2" Pellet	58167
1/2" Pellet, Irradiated	58170

**Other Forms Available By Request*

INGREDIENTS

Lard	28.3150
Casein - Vitamin Free	26.7600
Dextrin	18.9872
Sucrose	6.3715
RP Mineral Mix #10 (adds 1.29% fiber)	6.3715
Corn Oil	6.3715
RP Vitamin Mix (adds 1.94% sucrose)	2.5486
Powdered Cellulose	1.9114
Inulin	1.9114
Choline Chloride	0.2548
DL-Methionine	0.1911
Blue Dye	0.0060

Part of the TestDiet® DIO Series

Basal Purified Diet with 12% Energy From Fat
- 1/2" Pellet - Catalog # 58145 (58G7)
- 1/2" Pellet, Irradiated - Catalog # 58168 (58G7)
- Meal - Catalog # 1810736 (58G7)
- Meal, Irradiated - Catalog # 1810737 (58G7)

Basal Purified Diet with 45% Energy From Fat
- 1/2" Pellet - Catalog # 58146 (58G8)
- Meal - Catalog # 58169 (58G8)

Basal Purified Diet with 70% Energy From Fat
- Meal - Catalog # 58171 (58H0)

FEEDING DIRECTIONS

Feed ad libitum. Plenty of fresh, clean water should be available at all times.

CAUTION:
Perishable, refrigerate upon receipt.

For experimental use only.

6/28/2005

NUTRITIONAL PROFILE [1]

Protein, %	***24.2***
Arginine, %	0.94
Histidine, %	0.69
Isoleucine, %	1.28
Leucine, %	2.31
Lysine, %	1.94
Methionine, %	0.88
Cystine, %	0.10
Phenylalanine, %	1.28
Tyrosine, %	1.35
Threonine, %	1.03
Tryptophan, %	0.30
Valine, %	1.53
Alanine, %	0.74
Aspartic Acid, %	1.72
Glutamic Acid, %	5.46
Glycine, %	0.52
Proline, %	3.15
Serine, %	1.48
Taurine, %	0.00
Fat, %	***34.7***
Cholesterol, ppm	269
Linoleic Acid, %	6.37
Linolenic Acid, %	0.18
Arachidonic Acid, %	0.05
Omega-3 Fatty Acids, %	0.18
Total Saturated Fatty Acids, %	12.62
Total Monounsaturated Fatty Acids, %	13.46
Fiber (max), %	***5.5***
Carbohydrates, %	***27.8***
Energy (kcal/g) [2]	***5.21***

From:	kcal	%
Protein	0.969	18.6
Fat (ether extract)	3.122	59.9
Carbohydrates	1.113	21.4

Minerals	
Calcium, %	0.77
Phosphorus, %	0.72
Phosphorus (available), %	0.72
Potassium, %	0.51
Magnesium, %	0.09
Sodium, %	0.27
Chlorine, %	0.30
Fluorine, ppm	6.3
Iron, ppm	80
Zinc, ppm	35
Manganese, ppm	83
Copper, ppm	19.1
Cobalt, ppm	4.1
Iodine, ppm	0.73
Chromium, ppm	3.9
Molybdenum, ppm	1.04
Selenium, ppm	0.29
Vitamins	
Vitamin A, IU/g	28.2
Vitamin D-3 (added), IU/g	2.8
Vitamin E, IU/kg	63.8
Vitamin K (as menadione), ppm	13.25
Thiamin Hydrochloride, ppm	26.3
Riboflavin, ppm	25.5
Niacin, ppm	115
Pantothenic Acid, ppm	70
Folic Acid, ppm	5.1
Pyridoxine, ppm	21.0
Biotin, ppm	0.5
Vitamin B-12, mcg/kg	25
Choline Chloride, ppm	1,784
Ascorbic Acid, ppm	0.0

1. Based on the latest ingredient analysis information. Since nutrient composition of natural ingredients varies, analysis will differ accordingly. Nutrients expressed as percent of ration on an As Fed basis except where otherwise indicated.
2. Energy (kcal/gm) - Sum of decimal fractions of protein, fat and carbohydrate x 4,9,4 kcal/gm respectively.

TestDiet
www.testdiet.com

References

Akter K, Lanza EA, Martin SA, Myronyuk N, Rua M, Raffa RB (2011) Diabetes mellitus and Alzheimer's disease: shared pathology and treatment? British Journal of Clinical Pharmacology 71:365-376.

Alzheimer's Association Report (2014) 2014 Alzheimer's disease facts and figures. Alzheimer's & Dementia 10:e47-e92.

Anstey KJ, Cherbuin N, Budge M, Young J (2011) Body mass index in midlife and late-life as a risk factor for dementia: a meta-analysis of prospective studies. Obesity Reviews 12:e426-e437.

Anstey KJ, Kingston A, Kiely KM, Luszcz MA, Mitchell P, Jagger C (2014) The influence of smoking, sedentary lifestyle and obesity on cognitive impairment-free life expectancy. International Journal of Epidemiology.

Arnold SE, Lucki I, Brookshire BR, Carlson GC, Browne CA, Kazi H, Bang S, Choi B-R, Chen Y, McMullen MF, Kim SF (2014) High fat diet produces brain insulin resistance, synaptodendritic abnormalities and altered behavior in mice. Neurobiology of Disease 67:79-87.

Arvanitakis Z, Wilson RS, Bienias JL, Evans DA, Bennett DA (2004) DIabetes mellitus and risk of alzheimer disease and decline in cognitive function. Archives of Neurology 61:661-666.

Bailey TL, Rivara CB, Rocher AB, Hof PR (2004) The nature and effects of cortical microvascular pathology in aging and Alzheimer's disease. Neurological Research 26:573-578.

Balakrishnan K, Verdile G, Mehta PD, Beilby J, Nolan D, Galvão DA, Newton R, Gandy SE, Martins RN (2005) Plasma Aβ42 correlates positively with increased body fat in healthy individuals. Journal of Alzheimer's Disease 8:269-282.

Balistreri CR, Caruso C, Candore G (2010) The Role of Adipose Tissue and Adipokines in Obesity-Related Inflammatory Diseases. Mediators of Inflammation 2010:802078.

Banks WA (2010) Mouse Models of Neurological Disorders: A View From the Blood-brain Barrier. Biochimica et biophysica acta 1802:881-888.

Banks WA, Burney BO, Robinson SM (2008) Effects of Triglycerides, Obesity, and Starvation on Ghrelin Transport Across the Blood-Brain Barrier. Peptides 29:2061-2065.

Banks WA, Coon AB, Robinson SM, Moinuddin A, Shultz JM, Nakaoke R, Morley JE (2004) Triglycerides Induce Leptin Resistance at the Blood-Brain Barrier. Diabetes 53:1253-1260.

Banks WA, Jaspan JB, Kastin AJ (1997) Selective, Physiological Transport of Insulin Across the Blood-Brain Barrier: Novel Demonstration by Species-Specific Radioimmunoassays. Peptides 18:1257-1262.

Banks WA, Robinson SM, Verma S, Morley JE (2003) Efflux of human and mouse amyloid β proteins 1–40 and 1–42 from brain: impairment in a mouse model of alzheimer's disease. Neuroscience 121:487-492.

Barger JL, Kayo T, Vann JM, Arias EB, Wang J, Hacker TA, Wang Y, Raederstorff D, Morrow JD, Leeuwenburgh C, Allison DB, Saupe KW, Cartee GD, Weindruch R, Prolla TA (2008) A Low Dose of Dietary Resveratrol Partially Mimics Caloric Restriction and Retards Aging Parameters in Mice. PLoS ONE 3:e2264.

Barron AM, Rosario ER, Elteriefi R, Pike CJ (2013) Sex-Specific Effects of High Fat Diet on Indices of Metabolic Syndrome in 3xTg-AD Mice: Implications for Alzheimer's Disease. PLoS ONE 8:e78554.

Bateman RJ, Xiong C, Benzinger TLS, Fagan AM, Goate A, Fox NC, Marcus DS, Cairns NJ, Xie X, Blazey TM, Holtzman DM, Santacruz A, Buckles V, Oliver A, Moulder K, Aisen PS, Ghetti B, Klunk WE, McDade E, Martins RN, Masters CL, Mayeux R, Ringman JM, Rossor MN, Schofield PR, Sperling RA, Salloway S, Morris JC, for the Dominantly Inherited Alzheimer N (2012) Clinical and Biomarker Changes in Dominantly Inherited Alzheimer's Disease. N Engl J Med 367:795-804.

Bell RD, Zlokovic BV (2009) Neurovascular mechanisms and blood–brain barrier disorder in Alzheimer's disease. Acta Neuropathol 118:103-113.

Benedict C, Brooks SJ, Kullberg J, Burgos J, Kempton MJ, Nordenskjöld R, Nylander R, Kilander L, Craft S, Larsson E-M, Johansson L, Ahlström H, Lind L, Schiöth HB (2012) Impaired Insulin Sensitivity as Indexed by the HOMA Score Is Associated With Deficits in Verbal Fluency and Temporal Lobe Gray Matter Volume in the Elderly. Diabetes Care 35:488-494.

Benedict C, Hallschmid M, Schultes B, Born J, Kern W (2007) Intranasal Insulin to Improve Memory Function in Humans. Neuroendocrinology 86:136-142.

Benilova I, Karran E, De Strooper B (2012) The toxic A[beta] oligomer and Alzheimer's disease: an emperor in need of clothes. Nat Neurosci 15:349-357.

Benoit M, Berrut G, Doussaint J, Bakchine S, Bonin-Guillaume S, Frémont P, Gallarda T, Krolak-Salmon P, Marquet T, Mékiès C, Sellal F, Schuck S, David R, Robert P (2012) Apathy and Depression in Mild

Alzheimer's Disease: A Cross-Sectional Study Using Diagnostic Criteria. Journal of Alzheimer's Disease 31:325-334.

Berridge MJ (2014) Calcium regulation of neural rhythms, memory and Alzheimer's disease. The Journal of Physiology 592:281-293.

Beydoun MA, Lhotsky A, Wang Y, Forno GD, An Y, Metter EJ, Ferrucci L, O'Brien R, Zonderman AB (2008) Association of Adiposity Status and Changes in Early to Mid-Adulthood With Incidence of Alzheimer's Disease. American Journal of Epidemiology 168:1179-1189.

Bhat NR (2010) Linking cardiometabolic disorders to sporadic Alzheimer's disease: a perspective on potential mechanisms and mediators. Journal of Neurochemistry 115:551-562.

Bidzan L, Bidzan M, Pąchalska M (2012) Aggressive and impulsive behavior in Alzheimer's disease and progression of dementia. Medical Science Monitor : International Medical Journal of Experimental and Clinical Research 18:CR182-CR189.

Bird TD (2014) Alzheimer Disease Overview. GeneReviews®

Biron KE, Dickstein DL, Gopaul R, Jefferies WA (2011) Amyloid Triggers Extensive Cerebral Angiogenesis Causing Blood Brain Barrier Permeability and Hypervascularity in Alzheimer's Disease. PLoS ONE 6:e23789.

Bitan G, Kirkitadze MD, Lomakin A, Vollers SS, Benedek GB, Teplow DB (2003) Amyloid β-protein (Aβ) assembly: Aβ40 and Aβ42 oligomerize through distinct pathways. Proceedings of the National Academy of Sciences of the United States of America 100:330-335.

Blass JP, Baker AC, Ko LW, Black RS (1990) Induction of Alzheimer antigens by an uncoupler of oxidative phosphorylation. Archives of Neurology 47:864-869.

Blázquez E, Velázquez E, Hurtado-Carneiro V, Ruiz-Albusac JM (2014a) Insulin in the Brain: Its Pathophysiological Implications for States Related with Central Insulin Resistance, Type 2 Diabetes and Alzheimer's Disease. Frontiers in Endocrinology 5:161.

Blázquez G, Cañete T, Tobeña A, Giménez-Llort L, Fernández-Teruel A (2014b) Cognitive and emotional profiles of aged Alzheimer's disease (3xTgAD) mice: Effects of environmental enrichment and sexual dimorphism. Behavioural Brain Research 268:185-201.

Blennow K, de Leon MJ, Zetterberg H (2006) Alzheimer's disease. The Lancet 368:387-403.

Boitard C, Etchamendy N, Sauvant J, Aubert A, Tronel S, Marighetto A, Layé S, Ferreira G (2012) Juvenile, but not adult exposure to high-fat diet impairs relational memory and hippocampal neurogenesis in mice. Hippocampus 22:2095-2100.

Borchelt DR, Thinakaran G, Eckman CB, Lee MK, Davenport F, Ratovitsky T, Prada C-M, Kim G, Seekins S, Yager D, Slunt HH, Wang R, Seeger M, Levey AI, Gandy SE, Copeland NG, Jenkins NA, Price DL, Younkin SG, Sisodia SS (1996) Familial Alzheimer's Disease–Linked Presenilin 1 Variants Elevate Aβ1–42/1–40 Ratio In Vitro and In Vivo. Neuron 17:1005-1013.

Bourasset F, Ouellet M, Tremblay C, Julien C, Do TM, Oddo S, LaFerla F, Calon F (2009) Reduction of the cerebrovascular volume in a transgenic mouse model of Alzheimer's disease. Neuropharmacology 56:808-813.

Bourlier V, Zakaroff-Girard A, Miranville A, De Barros S, Maumus M, Sengenes C, Galitzky J, Lafontan M, Karpe F, Frayn KN, Bouloumié A (2008) Remodeling Phenotype of Human Subcutaneous Adipose Tissue Macrophages. Circulation 117:806-815.

Bournat JC, Brown CW (2010) Mitochondrial dysfunction in obesity. Current Opinion in Endocrinology, Diabetes and Obesity 17:446-452.

Broadbent NJ, Squire LR, Clark RE (2004) Spatial memory, recognition memory, and the hippocampus. Proceedings of the National Academy of Sciences of the United States of America 101:14515-14520.

Brooks SJ, Benedict C, Burgos J, Kempton MJ, Kullberg J, Nordenskjold R, Kilander L, Nylander R, Larsson EM, Johansson L, Ahlstrom H, Lind L, Schioth HB (2013) Late-life obesity is associated with smaller global and regional gray matter volumes: a voxel-based morphometric study. Int J Obes 37:230-236.

Bruce-Keller AJ, Keller JN, Morrison CD (2009) Obesity and vulnerability of the CNS. Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease 1792:395-400.

Butterfield DA, Di Domenico F, Barone E (2014) Elevated risk of type 2 diabetes for development of Alzheimer disease: A key role for oxidative stress in brain. Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease 1842:1693-1706.

Calkins M, Manczak M, Reddy P (2012) Mitochondria-Targeted Antioxidant SS31 Prevents Amyloid Beta-Induced Mitochondrial Abnormalities and Synaptic Degeneration in Alzheimer’s Disease. Pharmaceuticals 5:1103-1119.

Calkins MJ, Reddy PH (2011) Amyloid beta impairs mitochondrial anterograde transport and degenerates synapses in Alzheimer's disease neurons. Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease 1812:507-513.

Camer D, Yu Y, Szabo A, Fernandez F, Dinh CHL, Huang X-F (2015) Bardoxolone methyl prevents high-fat diet-induced alterations in prefrontal cortex signalling molecules involved in recognition memory. Progress in Neuro-Psychopharmacology and Biological Psychiatry 59:68-75.

Cannon B, Nedergaard J (2004) Brown Adipose Tissue: Function and Physiological Significance.

Carey AN, Gomes SM, Shukitt-Hale B (2014) Blueberry Supplementation Improves Memory in Middle-Aged Mice Fed a High-Fat Diet. J Agric Food Chem 62:3972-3978.

Carlson GA (2003) A welcoming environment for amyloid plaques. Nat Neurosci 6:328-330.

Carow, B. and M. E. Rottenberg (2014). "SOCS3, a Major Regulator of Infection and Inflammation." Frontiers in Immunology 5: 58.

Carson JA, Turner AJ (2002) β-Amyloid catabolism: roles for neprilysin (NEP) and other metallopeptidases? Journal of Neurochemistry 81:1-8.

Caspersen C, Wang N, Yao J, Sosunov A, Chen X, Lustbader JW, Xu HW, Stern D, McKhann G, Yan SD (2005) Mitochondrial Aβ: a potential focal point for neuronal metabolic dysfunction in Alzheimer’s disease. The FASEB Journal 19:2040-2041.

Chang H-C, Tai Y-T, Cherng Y-G, Lin J-W, Liu S-H, Chen T-L, Chen R-M (2014) Resveratrol Attenuates High-Fat Diet-Induced Disruption of the Blood–Brain Barrier and Protects Brain Neurons from Apoptotic Insults. J Agric Food Chem 62:3466-3475.

Chen Y, Zhao Y, Dai C-l, Liang Z, Run X, Iqbal K, Liu F, Gong C-X (2014) Intranasal insulin restores insulin signaling, increases synaptic proteins, and reduces Aβ level and microglia activation in the brains of 3xTg-AD mice. Exp Neurol 261:610-619.

Cheng A, Hou Y, Mattson MP (2010) Mitochondria and Neuroplasticity. ASN Neuro 2.

Chiu S-L, Chen C-M, Cline HT (2008) Insulin Receptor Signaling Regulates Synapse Number, Dendritic Plasticity and Circuit Function in Vivo. Neuron 58:708-719.

Choi YK, Kim K-W (2008) Blood-neural barrier: its diversity and coordinated cell-to-cell communication. BMB reports 41:345-352.

Cholerton B, Baker LD, Craft S (2013) Insulin, cognition, and dementia. European Journal of Pharmacology 719:170-179.

Citron M (2010) Alzheimer's disease: strategies for disease modification. Nat Rev Drug Discov 9:387-398.

Claxton A, Baker LD, Hanson A, Trittschuh EH, Cholerton B, Morgan A, Callaghan M, Arbuckle M, Behl C, Craft S (2015) Long-Acting Intranasal Insulin Detemir Improves Cognition for Adults with Mild Cognitive Impairment or Early-Stage Alzheimer's Disease Dementia. Journal of Alzheimer's Disease 44:897-906.

Cleary JP, Walsh DM, Hofmeister JJ, Shankar GM, Kuskowski MA, Selkoe DJ, Ashe KH (2005) Natural oligomers of the amyloid-[beta] protein specifically disrupt cognitive function. Nat Neurosci 8:79-84.

Clinton LK, Billings LM, Green KN, Caccamo A, Ngo J, Oddo S, McGaugh JL, LaFerla FM (2007) Age-dependent sexual dimorphism in cognition and stress response in the 3xTg-AD mice. Neurobiology of Disease 28:76-82.

Corder EH, Saunders AM, Strittmatter WJ, Schmechel DE, Gaskell PC, Small GW, Roses AD, Haines JL, Pericak-Vance MA (1993) Gene Dose of Apolipoprotein E Type 4 Allele and the Risk of Alzheimer's Disease in Late Onset Families. Science 261.

Corral-Debrinski M, Horton T, Lott MT, Shoffner JM, McKee AC, Beal MF, Graham BH, Wallace DC (1994) Marked Changes in Mitochondrial DNA Deletion Levels in Alzheimer Brains. Genomics 23:471-476.

Correia SC, Santos RX, Carvalho C, Cardoso S, Candeias E, Santos MS, Oliveira CR, Moreira PI (2012) Insulin signaling, glucose metabolism and mitochondria: Major players in Alzheimer's disease and diabetes interrelation. Brain Research 1441:64-78.

Coskun PE, Beal MF, Wallace DC (2004) Alzheimer's brains harbor somatic mtDNA control-region mutations that suppress mitochondrial transcription and replication. Proceedings of the National Academy of Sciences of the United States of America 101:10726-10731.

Craft S (2005) Insulin resistance syndrome and Alzheimer's disease: Age- and obesity-related effects on memory, amyloid, and inflammation. Neurobiology of Aging 26:65-69.

Craft S, Baker LD, Montine TJ, Minoshima S, Watson GS, Claxton A, Arbuckle M, Callaghan M, Tsai E, Plymate SR, Green PS, Leverenz J, Cross D, Gerton B (2012) Intranasal Insulin Therapy for Alzheimer Disease and Amnestic Mild Cognitive Impairment. Archives of Neurology 69:29-38.

Craft S, Peskind E, Schwartz MW, Schellenberg GD, Raskind M, Porte D (1998) Cerebrospinal fluid and plasma insulin levels in Alzheimer's disease: Relationship to severity of dementia and apolipoprotein E genotype. Neurology 50:164-168.

Crouch PJ, Blake R, Duce JA, Ciccotosto GD, Li Q-X, Barnham KJ, Curtain CC, Cherny RA, Cappai R, Dyrks T, Masters CL, Trounce IA (2005) Copper-Dependent Inhibition of Human Cytochrome c Oxidase by a Dimeric Conformer of Amyloid-β1-42. The Journal of Neuroscience 25:672-679.

Dandona P, Aljada A, Chaudhuri A, Mohanty P, Garg R (2005) Metabolic Syndrome: A Comprehensive Perspective Based on Interactions Between Obesity, Diabetes, and Inflammation. Circulation 111:1448-1454.

Davidson TL, Monnot A, Neal AU, Martin AA, Horton JJ, Zheng W (2012) The effects of a high-energy diet on hippocampal-dependent discrimination performance and blood–brain barrier integrity differ for diet-induced obese and diet-resistant rats. Physiology & Behavior 107:26-33.

Davis, K. E., A. Easton, et al. (2013a). "Episodic-Like Memory for What-Where-Which Occasion is Selectively Impaired in the 3xTgAD Mouse Model of Alzheimer's Disease." Journal of Alzheimer's Disease 33(3): 681-698.

Davis, K. E., M. J. Eacott, et al. (2013b). "Episodic-like memory is sensitive to both Alzheimer's-like pathological accumulation and normal ageing processes in mice." Behavioural Brain Research 254(0): 73-82.

De-Paula V, Radanovic M, Diniz BS, Forlenza OV (2012) Alzheimer's Disease. In: Protein Aggregation and Fibrillogenesis in Cerebral and Systemic Amyloid Disease, vol. 65 (Harris, J. R., ed), pp 329-352: Springer Netherlands.

De Felice FG, Ferreira ST (2014) Inflammation, Defective Insulin Signaling, and Mitochondrial Dysfunction as Common Molecular Denominators Connecting Type 2 Diabetes to Alzheimer Disease. Diabetes 63:2262-2272.

De Felice FG, Velasco PT, Lambert MP, Viola K, Fernandez SJ, Ferreira ST, Klein WL (2007) Aβ Oligomers Induce Neuronal Oxidative Stress through an N-Methyl-D-aspartate Receptor-dependent Mechanism That Is Blocked by the Alzheimer Drug Memantine. Journal of Biological Chemistry 282:11590-11601.

Deane R, Wu Z, Zlokovic BV (2004) RAGE (Yin) Versus LRP (Yang) Balance Regulates Alzheimer Amyloid β-Peptide Clearance Through Transport Across the Blood–Brain Barrier. Stroke 35:2628-2631.

Debette S, Seshadri S, Beiser A, Au R, Himali JJ, Palumbo C, Wolf PA, DeCarli C (2011) Midlife vascular risk factor exposure accelerates structural brain aging and cognitive decline. Neurology 77:461-468.

Deckers K, van Boxtel MPJ, Schiepers OJG, de Vugt M, Muñoz Sánchez JL, Anstey KJ, Brayne C, Dartigues J-F, Engedal K, Kivipelto M, Ritchie K, Starr JM, Yaffe K, Irving K, Verhey FRJ, Köhler S (2015) Target risk factors for dementia prevention: a systematic review and Delphi consensus study on the evidence from observational studies. International Journal of Geriatric Psychiatry 30:234-246.

del Campo A, Parra V, Vásquez-Trincado C, Gutiérrez T, Morales PE, López-Crisosto C, Bravo-Sagua R, Navarro-Marquez MF, Verdejo HE, Contreras-Ferrat A, Troncoso R, Chiong M, Lavandero S (2014) Mitochondrial fragmentation impairs insulin-dependent glucose uptake by modulating Akt activity through mitochondrial Ca2+ uptake.

Demetrius L, Magistretti PJ, Pellerin L (2015) Alzheimer's disease: the Amyloid hypothesis and the Inverse Warburg effect. Frontiers in Physiology 5.

Demuro A, Parker I, Stutzmann GE (2010) Calcium Signaling and Amyloid Toxicity in Alzheimer Disease. J Biol Chem 285:12463-12468.

Devi L, Prabhu BM, Galati DF, Avadhani NG, Anandatheerthavarada HK (2006) Accumulation of Amyloid Precursor Protein in the Mitochondrial Import Channels of Human Alzheimer's Disease Brain Is Associated with Mitochondrial Dysfunction. The Journal of Neuroscience 26:9057-9068.

Dickson DW, Crystal HA, Bevona C, Honer W, Vincent I, Davies P (1995) Correlations of synaptic and pathological markers with cognition of the elderly. Neurobiology of Aging 16:285-298.

Dickstein DL, Biron KE, Ujiie M, Pfeifer CG, Jeffries AR, Jefferies WA (2006) Aβ peptide immunization restores blood-brain barrier integrity in Alzheimer disease. The FASEB Journal 20:426-433.

Dineley KT, Jahrling JB, Denner L (2014) Insulin resistance in Alzheimer's disease. Neurobiology of Disease 72, Part A:92-103.

Ding F, Yao J, Rettberg JR, Chen S, Brinton RD (2013) Early Decline in Glucose Transport and Metabolism Precedes Shift to Ketogenic System in Female Aging and Alzheimer's Mouse Brain: Implication for Bioenergetic Intervention. PLoS ONE 8:e79977.

Do TM, Alata W, Dodacki A, Traversy M-T, Chacun H, Pradier L, Scherrmann J-M, Farinotti R, Calon F, Bourasset F (2014) Altered cerebral vascular volumes and solute transport at the blood–brain barriers of two transgenic mouse models of Alzheimer's disease. Neuropharmacology 81:311-317.

Donahue J, Flaherty S, Johanson C, Duncan J, III, Silverberg G, Miller M, Tavares R, Yang W, Wu Q, Sabo E, Hovanesian V, Stopa E (2006) RAGE, LRP-1, and amyloid-beta protein in Alzheimer's disease. Acta Neuropathol 112:405-415.

Du H, Guo L, Fang F, Chen D, Sosunov AA, McKhann GM, Yan Y, Wang C, Zhang H, Molkentin JD, Gunn-Moore FJ, Vonsattel JP, Arancio O, Chen JX, Yan SD (2008) Cyclophilin D deficiency attenuates mitochondrial and neuronal perturbation and ameliorates learning and memory in Alzheimer's disease. Nature Medicine 14:1097-1105.

Du H, Guo L, Yan S, Sosunov AA, McKhann GM, ShiDu Yan S (2010) Early deficits in synaptic mitochondria in an Alzheimer's disease mouse model. Proceedings of the National Academy of Sciences of the United States of America 107:18670-18675.

Duff K, Eckman C, Zehr C, Yu X, Prada C-M, Perez-tur J, Hutton M, Buee L, Harigaya Y, Yager D, Morgan D, Gordon MN, Holcomb L, Refolo L, Zenk B, Hardy J, Younkin S (1996) Increased amyloid-[beta]42(43) in brains of mice expressing mutant presenilin 1. Nature 383:710-713.

Elias MF, Beiser A, Wolf PA, Au R, White RF, D'Agostino RB (2000) The preclinical phase of alzheimer disease: A 22-year prospective study of the framingham cohort. Archives of Neurology 57:808-813.

Elias MF, Elias PK, Sullivan LM, Wolf PA, D'Agostino RB (2003) Lower cognitive function in the presence of obesity and hypertension: the Framingham heart study. Int J Obes Relat Metab Disord 27:260-268.

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

Erickson MA, Banks WA (2013) Blood-brain barrier dysfunction as a cause and consequence of Alzheimer/'s disease. J Cereb Blood Flow Metab 33:1500-1513.

Estadella D, #xe9, bora, da Penha Oller do Nascimento CM, Oyama LM, Ribeiro EB, #xe2, maso AR, de Piano A (2013) Lipotoxicity: Effects of Dietary Saturated and Transfatty Acids. Mediators of Inflammation 2013:13.

Evin G, Zhu A, Holsinger RMD, Masters CL, Li Q-X (2003) Proteolytic processing of the Alzheimer's disease amyloid precursor protein in brain and platelets. Journal of Neuroscience Research 74:386-392.

Farkas E, Luiten PGM (2001) Cerebral microvascular pathology in aging and Alzheimer's disease. Progress in Neurobiology 64:575-611.

Farris W, Mansourian S, Chang Y, Lindsley L, Eckman EA, Frosch MP, Eckman CB, Tanzi RE, Selkoe DJ, Guénette S (2003) Insulin-degrading enzyme regulates the levels of insulin, amyloid β-protein, and the β-amyloid precursor protein intracellular domain in vivo. Proceedings of the National Academy of Sciences 100:4162-4167.

Festa A, Williams K, D'Agostino R, Wagenknecht LE, Haffner SM (2006) The Natural Course of β-Cell Function in Nondiabetic and Diabetic Individuals: The Insulin Resistance Atherosclerosis Study. Diabetes 55:1114-1120.

Fitzpatrick AL, Kuller LH, Lopez OL, et al. (2009) Midlife and late-life obesity and the risk of dementia: Cardiovascular health study. Archives of Neurology 66:336-342.

Fotuhi M, Do D, Jack C (2012) Modifiable factors that alter the size of the hippocampus with ageing. Nature Reviews Neurology 8:189-202.

Freeman LR, Granholm A-CE (2012) Vascular changes in rat hippocampus following a high saturated fat and cholesterol diet. J Cereb Blood Flow Metab 32:643-653.

Freiherr J, Hallschmid M, Frey WH, Brünner YF, Chapman CD, Hölscher C, Craft S, De Felice FG, Benedict C (2013) Intranasal Insulin as a Treatment for Alzheimer's Disease: A Review of Basic Research and Clinical Evidence. CNS Drugs 27:505-514.

Frölich L, Blum-Degen D, Bernstein HG, Engelsberger S, Humrich J, Laufer S, Muschner D, Thalheimer A, Türk A, Hoyer S, Zöchling R, Boissl KW, Jellinger K, Riederer P (1998) Brain insulin and insulin receptors in aging and sporadic Alzheimer's disease. J Neural Transm 105:423-438.

Frolich L, Blum-Degen D, Riederer P, Hoyer S (1999) A Disturbance in the Neuronal Insulin Receptor Signal Transduction in Sporadic Alzheimer's Disease. Annals of the New York Academy of Sciences 893:290-293.

Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, Nakayama O, Makishima M, Matsuda M, Shimomura I (2004) Increased oxidative stress in obesity and its impact on metabolic syndrome. The Journal of Clinical Investigation 114:1752-1761.

Gabuzda D, Busciglio J, Chen LB, Matsudaira P, Yankner BA (1994) Inhibition of energy metabolism alters the processing of amyloid precursor protein and induces a potentially amyloidogenic derivative. Journal of Biological Chemistry 269:13623-13628.

Garcia-Escudero V, Martin-Maestro P, Perry G, Avila J (2013) Deconstructing Mitochondrial Dysfunction in Alzheimer Disease. Oxidative Medicine and Cellular Longevity 2013:13.

Gasparini L, Gouras GK, Wang R, Gross RS, Beal MF, Greengard P, Xu H (2001) Stimulation of β-amyloid precursor protein trafficking by insulin reduces intraneuronal β-amyloid and requires mitogen-activated protein kinase signaling. Journal of Neuroscience 21:2561-2570.

Gasparini L, Racchi M, Benussi L, Curti D, Binetti G, Bianchetti A, Trabucchi M, Govoni S (1997) Effect of energy shortage and oxidative stress on amyloid precursor protein metabolism in COS cells. Neuroscience Letters 231:113-117.

Gazdzinski S, Kornak J, Weiner MW, Meyerhoff DJ (2008) Body mass index and magnetic resonance markers of brain integrity in adults. Annals of Neurology 63:652-657.

Gazdzinski S, Millin R, Kaiser LG, Durazzo TC, Mueller SG, Weiner MW, Meyerhoff DJ (2010) BMI and neuronal integrity in healthy, cognitively normal elderly: A proton magnetic resonance spectroscopy study. Obesity 18:743-748.

George AJ, Holsinger RMD, McLean CA, Laughton KM, Beyreuther K, Evin G, Masters CL, Li Q-X (2004) APP intracellular domain is increased and soluble Aβ is reduced with diet-induced hypercholesterolemia in a transgenic mouse model of Alzheimer disease. Neurobiology of Disease 16:124-132.

Gesta S, Tseng Y-H, Kahn CR (2007) Developmental Origin of Fat: Tracking Obesity to Its Source. Cell 131:242-256.

Ghasemi R, Dargahi L, Haeri A, Moosavi M, Mohamed Z, Ahmadiani A (2013) Brain Insulin Dysregulation: Implication for Neurological and Neuropsychiatric Disorders. Mol Neurobiol 47:1045-1065.

Glenner GG, Wong CW (1984) Alzheimer's disease and Down's syndrome: Sharing of a unique cerebrovascular amyloid fibril protein. Biochemical and Biophysical Research Communications 122:1131-1135.

Goate A, Chartier-Harlin M-C, Mullan M, Brown J, Crawford F, Fidani L, Giuffra L, Haynes A, Irving N, James L, Mant R, Newton P, Rooke K, Roques P, Talbot C, Pericak-Vance M, Roses A, Williamson R, Rossor M, Owen M, Hardy J (1991) Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. Nature 349:704-706.

Gold SM, Dziobek I, Sweat V, Tirsi A, Rogers K, Bruehl H, Tsui W, Richardson S, Javier E, Convit A (2007) Hippocampal damage and memory impairments as possible early brain complications of type 2 diabetes. Diabetologia 50:711-719.

Gräff J, Kahn M, Samiei A, Gao J, Ota KT, Rei D, Tsai L-H (2013) A Dietary Regimen of Caloric Restriction or Pharmacological Activation of SIRT1 to Delay the Onset of Neurodegeneration. The Journal of Neuroscience 33:8951-8960.

Grammas P (2011) Neurovascular dysfunction, inflammation and endothelial activation: Implications for the pathogenesis of Alzheimer's disease. Journal of Neuroinflammation 8:26-26.

Grammas P, Yamada M, Zlokovic B (2002) The cerebromicrovasculature: A key player in the pathogenesis of Alzheimer's disease. Journal of Alzheimer's Disease 4:217-223.

Gregor MF, Hotamisligil GS (2011) Inflammatory Mechanisms in Obesity. Annual Review of Immunology 29:415-445.

Grossi D, Santangelo G, Barbarulo AM, Vitale C, Castaldo G, Proto MG, Siano P, Barone P, Trojano L (2013) Apathy and related executive syndromes in dementia associated with Parkinson's disease and in Alzheimer's disease. Behavioural Neurology 27:515-522.

Guri AJ, Bassaganya-Riera J (2011) Systemic Effects of White Adipose Tissue Dysregulation and Obesity-Related Inflammation. Obesity 19:689-700.

Gustafson D, Lissner L, Bengtsson C, Björkelund C, Skoog I (2004) A 24-year follow-up of body mass index and cerebral atrophy. Neurology 63:1876-1881.

Gustafson D, Rothenberg E, Blennow K, Steen B, Skoog I (2003) AN 18-year follow-up of overweight and risk of alzheimer disease. Archives of Internal Medicine 163:1524-1528.

Gustafson DR (2012) Adiposity and cognitive decline: underlying mechanisms. J Alzheimers Dis 30 Suppl 2:S97-112.

Haass C, Selkoe D (2007) Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid beta-peptide. Nat Rev Mol Cell Biol 8:101 - 112.

Haj-ali V, Mohaddes G, Babri SH (2009) Intracerebroventricular insulin improves spatial learning and memory in male Wistar rats. Behavioral neuroscience 123:1309-1314.

Halagappa VKM, Guo Z, Pearson M, Matsuoka Y, Cutler RG, LaFerla FM, Mattson MP (2007) Intermittent fasting and caloric restriction ameliorate age-related behavioral deficits in the triple-transgenic mouse model of Alzheimer's disease. Neurobiology of Disease 26:212-220.

Hansson Petersen CA, Alikhani N, Behbahani H, Wiehager B, Pavlov PF, Alafuzoff I, Leinonen V, Ito A, Winblad B, Glaser E, Ankarcrona M (2008) The amyloid β-peptide is imported into mitochondria via the TOM import machinery and localized to mitochondrial cristae. Proceedings of the National Academy of Sciences of the United States of America 105:13145-13150.

Haohao Z, Guijun Q, Juan Z, Wen K, Lulu C (2015) Resveratrol improves high-fat diet induced insulin resistance by rebalancing subsarcolemmal mitochondrial oxidation and antioxidantion. J Physiol Biochem 71:121-131.

Hardy J (2009) The amyloid hypothesis for Alzheimer’s disease: a critical reappraisal. Journal of Neurochemistry 110:1129-1134.

Hardy J, Higgins GA (1992) Alzheimer's disease: the amyloid cascade hypothesis. Science 256:184-185.

Hardy J, Selkoe DJ (2002) The Amyloid Hypothesis of Alzheimer's Disease: Progress and Problems on the Road to Therapeutics. Science 297:353-356.

Harper JD, Wong SS, Lieber CM, Lansbury Jr PT (1997) Observation of metastable Aβ amyloid protofibrils by atomic force microscopy. Chemistry & Biology 4:119-125.

Haslam DW, James WPT (2005) Obesity. The Lancet 366:1197-1209.

Hassing LB, Dahl AK, Thorvaldsson V, Berg S, Gatz M, Pedersen NL, Johansson B (2009) Overweight in midlife and risk of dementia: a 40-year follow-up study. Int J Obes 33:893-898.

Henegar C, Tordjman J, Achard V, Lacasa D, Cremer I, Guerre-Millo M, Poitou C, Basdevant A, Stich V, Viguerie N, Langin D, Bedossa P, Zucker J-D, Clement K (2008) Adipose tissue transcriptomic signature highlights the pathological relevance of extracellular matrix in human obesity. Genome Biol 9:R14-R14.

Heni M, Schöpfer P, Peter A, Sartorius T, Fritsche A, Synofzik M, Häring H-U, Maetzler W, Hennige A (2014) Evidence for altered transport of insulin across the blood–brain barrier in insulin-resistant humans. Acta Diabetol 51:679-681.

Heyward FD, Walton RG, Carle MS, Coleman MA, Garvey WT, Sweatt JD (2012) Adult mice maintained on a high-fat diet exhibit object location memory deficits and reduced hippocampal SIRT1 gene expression. Neurobiology of Learning and Memory 98:25-32.

Hirabara SM, Gorjão R, Vinolo MA, Rodrigues AC, Nachbar RT, Curi R (2012) Molecular Targets Related to Inflammation and Insulin Resistance and Potential Interventions. Journal of Biomedicine and Biotechnology 2012:379024.

Hirai K, Aliev G, Nunomura A, Fujioka H, Russell RL, Atwood CS, Johnson AB, Kress Y, Vinters HV, Tabaton M, Shimohama S, Cash AD, Siedlak SL, Harris PLR, Jones PK, Petersen RB, Perry G, Smith MA (2001) Mitochondrial Abnormalities in Alzheimer's Disease. The Journal of Neuroscience 21:3017-3023.

Hirata-Fukae C, Li H-F, Hoe H-S, Gray AJ, Minami SS, Hamada K, Niikura T, Hua F, Tsukagoshi-Nagai H, Horikoshi-Sakuraba Y, Mughal M, Rebeck GW, LaFerla FM, Mattson MP, Iwata N, Saido TC, Klein WL, Duff KE, Aisen PS, Matsuoka Y (2008) Females exhibit more extensive amyloid, but not tau, pathology in an Alzheimer transgenic model. Brain Research 1216:92-103.

Ho L, Qin W, Pompl PN, Xiang Z, Wang J, Zhao Z, Peng Y, Cambareri G, Rocher A, Mobbs CV, Hof PR, Pasinetti GM (2004) Diet-induced insulin resistance promotes amyloidosis in a transgenic mouse model of Alzheimer's disease. The FASEB Journal.

Hohsfield LA, Daschil N, Orädd G, Strömberg I, Humpel C (2014) Vascular pathology of 20-month-old hypercholesterolemia mice in comparison to triple-transgenic and APPSwDI Alzheimer's disease mouse models. Molecular and Cellular Neuroscience 63:83-95.

Holscher C (2014) Insulin, incretins and other growth factors as potential novel treatments for Alzheimer’s and Parkinson’s diseases. Biochemical Society Transactions 42:593–599.

Hölscher C First clinical data of the neuroprotective effects of nasal insulin application in patients with Alzheimer's disease. Alzheimer's & Dementia: The Journal of the Alzheimer's Association 10:S33-S37.

Hölscher C (2011) Diabetes as a risk factor for Alzheimer's disease: insulin signalling impairment in the brain as an alternative model of Alzheimer's disease. Biochemical Society Transactions 39:891-897.

Holtzman DM, Fagan AM, Mackey B, Tenkova T, Sartorius L, Paul SM, Bales K, Ashe KH, Irizarry MC, Hyman BT (2000) Apolipoprotein E facilitates neuritic and cerebrovascular plaque formation in an Alzheimer's disease model. Ann Neurology 47:739-747.

Hoshi M, Sato M, Matsumoto S, Noguchi A, Yasutake K, Yoshida N, Sato K (2003) Spherical aggregates of β-amyloid (amylospheroid) show high neurotoxicity and activate tau protein kinase I/glycogen synthase kinase-3β. Proceedings of the National Academy of Sciences of the United States of America 100:6370-6375.

Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM (1995) Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. Journal of Clinical Investigation 95:2409-2415.

Hotamisligil GS, Erbay E (2008) Nutrient sensing and inflammation in metabolic diseases. Nature reviews Immunology 8:923.

Houstis N, Rosen ED, Lander ES (2006) Reactive oxygen species have a causal role in multiple forms of insulin resistance. Nature 440:944-948.

Howard JK, Flier JS (2006) Attenuation of leptin and insulin signaling by SOCS proteins. Trends in Endocrinology & Metabolism 17:365-371.

Hsu TM, Kanoski SE (2014) Blood-brain barrier disruption: mechanistic links between Western diet consumption and dementia. Frontiers in Aging Neuroscience 6:88.

Huang H-C, Lin C-J, Liu W-J, Jiang R-R, Jiang Z-F (2011) Dual effects of curcumin on neuronal oxidative stress in the presence of Cu(II). Food and Chemical Toxicology 49:1578-1583.

Hwang L-L, Wang C-H, Li T-L, Chang S-D, Lin L-C, Chen C-P, Chen C-T, Liang K-C, Ho I-K, Yang W-S, Chiou L-C (2010) Sex Differences in High-fat Diet-induced Obesity, Metabolic Alterations and Learning, and Synaptic Plasticity Deficits in Mice. Obesity 18:463-469.

Iadecola C (2010) The overlap between neurodegenerative and vascular factors in the pathogenesis of dementia. Acta Neuropathol 120:287-296.

Iadecola C, Zhang F, Niwa K, Eckman C, Turner SK, Fischer E, Younkin S, Borchelt DR, Hsiao KK, Carlson GA (1999) SOD1 rescues cerebral endothelial dysfunction in mice overexpressing amyloid precursor protein. Nat Neurosci 2:157-161.

Ichimura H, Parthasarathi K, Quadri S, Issekutz AC, Bhattacharya J (2003) Mechano-oxidative coupling by mitochondria induces proinflammatory responses in lung venular capillaries. The Journal of Clinical Investigation 111:691-699.

Jacobsen JS, Reinhart P, Pangalos M (2005) Current concepts in therapeutic strategies targeting cognitive decline and disease modification in Alzheimer's disease. Neurotherapeutics 2:612-626.

Jahangiri A, Wilson PG, Hou T, Brown A, King VL, Tannock LR (2013) SAA is Found on ApoB-Containing Lipoproteins in Obese Diabetic Humans. Obesity (Silver Spring, Md) 21:993-996.

Jang H, Arce FT, Ramachandran S, Capone R, Lal R, Nussinov R (2010a) β-Barrel Topology of Alzheimer's β-Amyloid Ion Channels. Journal of Molecular Biology 404:917-934.

Jang H, Teran Arce F, Ramachandran S, Capone R, Lal R, Nussinov R (2010b) Structural Convergence Among Diverse, Toxic β-Sheet Ion Channels. The Journal of Physical Chemistry B 114:9445-9451.

Jang H, Zheng J, Lal R, Nussinov R (2008) New structures help the modeling of toxic amyloidß ion channels. Trends in Biochemical Sciences 33:91-100.

Janson J, Laedtke T, Parisi JE, O'Brien P, Petersen RC, Butler PC (2004) Increased Risk of Type 2 Diabetes in Alzheimer Disease. Diabetes 53:474-481.

Jeon BT, Jeong EA, Shin HJ, Lee Y, Lee DH, Kim HJ, Kang SS, Cho GJ, Choi WS, Roh GS (2012) Resveratrol Attenuates Obesity-Associated Peripheral and Central Inflammation and Improves Memory Deficit in Mice Fed a High-Fat Diet. Diabetes 61:1444-1454.

Jernås M, Palming J, Sjöholm K, Jennische E, Svensson P-A, Gabrielsson BG, Levin M, Sjögren A, Rudemo M, Lystig TC, Carlsson B, Carlsson LMS, Lönn M (2006) Separation of human adipocytes by size: hypertrophic fat cells display distinct gene expression. The FASEB Journal 20:1540-1542.

Jin Z, Jin Y, Kumar-Mendu S, Degerman E, Groop L, Birnir B (2011) Insulin Reduces Neuronal Excitability by Turning on GABA_A Channels that Generate Tonic Current. PLoS ONE 6:e16188.

Jo J, Whitcomb DJ, Olsen KM, Kerrigan TL, Lo S-C, Bru-Mercier G, Dickinson B, Scullion S, Sheng M, Collingridge G, Cho K (2011) A[beta]1-42 inhibition of LTP is mediated by a signaling pathway involving caspase-3, Akt1 and GSK-3[beta]. Nat Neurosci 14:545-547.

Julien C, Tremblay C, Phivilay A, Berthiaume L, Émond V, Julien P, Calon F (2010) High-fat diet aggravates amyloid-beta and tau pathologies in the 3xTg-AD mouse model. Neurobiology of Aging 31:1516-1531.

Jurdak N, Kanarek RB (2009) Sucrose-induced obesity impairs novel object recognition learning in young rats. Physiology & Behavior 96:1-5.

Jurdak N, Lichtenstein AH, Kanarek RB (2008) Diet-induced obesity and spatial cognition in young male rats. Nutritional Neuroscience 11:48-54.

Kaczmarczyk MM, Machaj AS, Chiu GS, Lawson MA, Gainey SJ, York JM, Meling DD, Martin SA, Kwakwa KA, Newman AF, Woods JA, Kelley KW, Wang Y, Miller MJ, Freund GG (2013) Methylphenidate prevents high-fat diet (HFD)-induced learning/memory impairment in juvenile mice. Psychoneuroendocrinology 38:1553-1564.

Kalaria RN (1996) Cerebral vessels in ageing and Alzheimer's disease. Pharmacology & Therapeutics 72:193-214.

Kalaria RN (2010) Vascular basis for brain degeneration: faltering controls and risk factors for dementia.

Kandimalla KK, Curran GL, Holasek SS, Gilles EJ, Wengenack TM, Poduslo JF (2005) Pharmacokinetic Analysis of the Blood-Brain Barrier Transport of 125I-Amyloid β Protein 40 in Wild-Type and Alzheimer's Disease Transgenic Mice (APP,PS1) and Its Implications for Amyloid Plaque Formation. Journal of Pharmacology and Experimental Therapeutics 313:1370-1378.

Kanoski SE, Davidson TL (2011) Western diet consumption and cognitive impairment: Links to hippocampal dysfunction and obesity. Physiology & Behavior 103:59-68.

Kanoski SE, Zhang Y, Zheng W, Davidson TL (2010) The Effects of a High-Energy Diet on Hippocampal Function and Blood-Brain Barrier Integrity in the Rat. Journal of Alzheimer's Disease 21:207-219.

Karimi SA, Salehi I, Komaki A, Sarihi A, Zarei M, Shahidi S (2013) Effect of high-fat diet and antioxidants on hippocampal long-term potentiation in rats: An in vivo study. Brain Research 1539:1-6.

Karran E, Mercken M, Strooper BD (2011) The amyloid cascade hypothesis for Alzheimer's disease: an appraisal for the development of therapeutics. Nat Rev Drug Discov 10:698-712.

Keaney JF, Larson MG, Vasan RS, Wilson PWF, Lipinska I, Corey D, Massaro JM, Sutherland P, Vita JA, Benjamin EJ (2003) Obesity and Systemic Oxidative Stress: Clinical Correlates of Oxidative Stress in The Framingham Study. Arteriosclerosis, Thrombosis, and Vascular Biology 23:434-439.

Khachaturian AS, Zandi PP, Lyketsos CG, et al. (2006) Antihypertensive medication use and incident alzheimer disease: The cache county study. Archives of Neurology 63:686-692.

Kim J, Basak JM, Holtzman DM (2009) The Role of Apolipoprotein E in Alzheimer's Disease. Neuron 63:287-303.

Kim JA, Tran ND, Li Z, Yang F, Zhou W, Fisher MJ (2005) Brain endothelial hemostasis regulation by pericytes. J Cereb Blood Flow Metab 26:209-217.

Kissebah AH, Krakower GR (1994) Regional adiposity and morbidity.

Kivipelto M, Helkala E-L, Laakso MP, Hänninen T, Hallikainen M, Alhainen K, Soininen H, Tuomilehto J, Nissinen A (2001) Midlife vascular risk factors and Alzheimer's disease in later life: longitudinal, population based study.

Kivipelto M, Ngandu T, Fratiglioni L, et al. (2005) Obesity and vascular risk factors at midlife and the risk of dementia and alzheimer disease. Archives of Neurology 62:1556-1560.

Knight EM, Martins IVA, Gümüsgöz S, Allan SM, Lawrence CB (2014) High-fat diet-induced memory impairment in triple-transgenic Alzheimer's disease (3xTgAD) mice is independent of changes in amyloid and tau pathology. Neurobiology of Aging 35:1821-1832.

Knight EM, Verkhratsky A, Luckman SM, Allan SM, Lawrence CB (2012) Hypermetabolism in a triple-transgenic mouse model of Alzheimer's disease. Neurobiology of Aging 33:187-193.

Kohjima M, Sun Y, Chan L (2010) Increased Food Intake Leads to Obesity and Insulin Resistance in the Tg2576 Alzheimer's Disease Mouse Model. Endocrinology 151:1532-1540.

Kosari S, Badoer E, Nguyen JCD, Killcross AS, Jenkins TA (2012) Effect of western and high fat diets on memory and cholinergic measures in the rat. Behavioural Brain Research 235:98-103.

Kwak YT, Yang Y, Pyo SJ, Koo M-S (2014) Clinical characteristics according to depression screening tools in patients with Alzheimer's disease: View from self, caregiver-reported and drug-intervention pattern. Geriatrics & Gerontology International 14:660-666.

LaFerla FM, Oddo S (2005) Alzheimer's disease: Aβ, tau and synaptic dysfunction. Trends in Molecular Medicine 11:170-176.

Lalonde R (2002) The neurobiological basis of spontaneous alternation. Neuroscience & Biobehavioral Reviews 26:91-104.

Lambert MP, Barlow AK, Chromy BA, Edwards C, Freed R, Liosatos M, Morgan TE, Rozovsky I, Trommer B, Viola KL, Wals P, Zhang C, Finch CE, Krafft GA, Klein WL (1998) Diffusible, nonfibrillar ligands derived from Aβ(1–42) are potent central nervous system neurotoxins. Proceedings of the National Academy of Sciences of the United States of America 95:6448-6453.

LaRue B, Hogg E, Sagare A, Jovanovic S, Maness L, Maurer C, Deane R, Zlokovic BV (2004) Method for measurement of the blood–brain barrier permeability in the perfused mouse brain: application to amyloid-β peptide in wild type and Alzheimer's Tg2576 mice. Journal of Neuroscience Methods 138:233-242.

Launer LJ, Ross GW, Petrovitch H, Masaki K, Foley D, White LR, Havlik RJ (2000) Midlife blood pressure and dementia: the Honolulu–Asia aging study☆. Neurobiology of Aging 21:49-55.

Lazzari C, Kipanyula MJ, Agostini M, Pozzan T, Fasolato C (2015) Aβ42 oligomers selectively disrupt neuronal calcium release. Neurobiology of Aging 36:877-885.

Leboucher A, Laurent C, Fernandez-Gomez F-J, Burnouf S, Troquier L, Eddarkaoui S, Demeyer D, Caillierez R, Zommer N, Vallez E, Bantubungi K, Breton C, Pigny P, Buée-Scherrer V, Staels B, Hamdane M, Tailleux A, Buée L, Blum D (2013) Detrimental Effects of Diet-Induced Obesity on τ Pathology Are Independent of Insulin Resistance in τ Transgenic Mice. Diabetes 62:1681-1688.

Lee YH, Martin JM, Maple RL, Tharp WG, Pratley RE (2009) Plasma Amyloid-β Peptide Levels Correlate with Adipocyte Amyloid Precursor Protein Gene Expression in Obese Individuals. Neuroendocrinology 90:383-390.

Leibson CL, Rocca WA, Hanson VA, Cha R, Kokmen E, O'Brien PC, Palumbo PJ (1997) The Risk of Dementia among Persons with Diabetes Mellitus: A Population-Based Cohort Studya. Annals of the New York Academy of Sciences 826:422-427.

Levin-Allerhand JA, Lominska CE, Smith JD (2002) Increased amyloid-β levels in APPSWE transgenic mice treated chronically with a physiological high-fat high-cholesterol diet. Journal of Nutrition, Health and Aging 6:315-319.

Levy-Lahad E, Wasco W, Poorkaj P, Romano D, Oshima J, Pettingell W, Yu C, Jondro P, Schmidt S, Wang K, al. e (1995) Candidate gene for the chromosome 1 familial Alzheimer's disease locus. Science 269:973-977.

Li J-c, Han L, Wen Y-x, Yang Y-x, Li S, Li X-s, Zhao C-j, Wang T-y, Chen H, Liu Y, Qi C-l, He X-d, Gu Q-l, Ye Y-x, Zhang Y, Huang R, Wu Y-e, He R-r, Kurihara H, Song X-y, Cao L, Wang L-j (2015) Increased Permeability of the Blood-Brain Barrier and Alzheimer's Disease-Like Alterations in Slit-2 Transgenic Mice. Journal of Alzheimer's Disease 43:535-548.

Li S, Hong S, Shepardson NE, Walsh DM, Shankar GM, Selkoe D (2009) Soluble oligomers of amyloid β-protein facilitate hippocampal long-term depression by disrupting neuronal glutamate uptake. Neuron 62:788-801.

Li W, Prakash R, Chawla D, Du W, Didion SP, Filosa JA, Zhang Q, Brann DW, Lima VV, Tostes RC, Ergul A (2013) Early effects of high-fat diet on neurovascular function and focal ischemic brain injury.

Lindwall G, Cole RD (1984) Phosphorylation affects the ability of tau protein to promote microtubule assembly. Journal of Biological Chemistry 259:5301-5305.

Luchsinger JA, Tang M-X, Stern Y, Shea S, Mayeux R (2001) Diabetes Mellitus and Risk of Alzheimer's Disease and Dementia with Stroke in a Multiethnic Cohort. American Journal of Epidemiology 154:635-641.

Lumeng CN, DeYoung SM, Bodzin JL, Saltiel AR (2007) Increased Inflammatory Properties of Adipose Tissue Macrophages Recruited During Diet-Induced Obesity. Diabetes 56:16-23.

Lustbader JW, Cirilli M, Lin C, Xu HW, Takuma K, Wang N, Caspersen C, Chen X, Pollak S, Chaney M, Trinchese F, Liu S, Gunn-Moore F, Lue L-F, Walker DG, Kuppusamy P, Zewier ZL, Arancio O, Stern D, Yan SS, Wu H (2004) ABAD Directly Links Aß to Mitochondrial Toxicity in Alzheimer's Disease. Science 304:448-452.

Lynch CM, Kinzenbaw DA, Chen X, Zhan S, Mezzetti E, Filosa J, Ergul A, Faulkner JL, Faraci FM, Didion SP (2013) Nox2-Derived Superoxide Contributes to Cerebral Vascular Dysfunction in Diet-Induced Obesity. Stroke 44:3195-3201.

Ma L, Wang J, Li Y (2015) Insulin resistance and cognitive dysfunction. Clinica Chimica Acta 444:18-23.

Ma W, Yuan L, Yu H, Xi Y, Xiao R (2014) Mitochondrial dysfunction and oxidative damage in the brain of diet-induced obese rats but not in diet-resistant rats. Life Sciences 110:53-60.

Mackic JB, Stins M, McComb JG, Calero M, Ghiso J, Kim KS, Yan SD, Stern D, Schmidt AM, Frangione B, Zlokovic BV (1998) Human blood-brain barrier receptors for Alzheimer's amyloid-beta 1- 40. Asymmetrical binding, endocytosis, and transcytosis at the apical side of brain microvascular endothelial cell monolayer. Journal of Clinical Investigation 102:734-743.

Maesako M, Uemura K, Kubota M, Kuzuya A, Sasaki K, Asada M, Watanabe K, Hayashida N, Ihara M, Ito H, Shimohama S, Kihara T, Kinoshita A (2012) Environmental enrichment ameliorated high-fat diet-induced Aβ deposition and memory deficit in APP transgenic mice. Neurobiology of Aging 33:1011.e1011-1011.e1023.

Manczak M, Anekonda TS, Henson E, Park BS, Quinn J, Reddy PH (2006) Mitochondria are a direct site of Aβ accumulation in Alzheimer's disease neurons: implications for free radical generation and oxidative damage in disease progression. Human Molecular Genetics 15:1437-1449.

Manczak M, Calkins MJ, Reddy PH (2011) Impaired mitochondrial dynamics and abnormal interaction of amyloid beta with mitochondrial protein Drp1 in neurons from patients with Alzheimer's disease: implications for neuronal damage. Human Molecular Genetics 20:2495-2509.

Manczak M, Mao P, Calkins M, Cornea A, Arubala RP, Murphy MP, Szeto HH, Park B, Reddy PH (2010) Mitochondria-targeted antioxidants protect against Abeta toxicity in Alzheimer's disease neurons. J Alzheimers Dis 20:S609-S631.

Manczak M, Reddy PH (2012) Abnormal interaction between the mitochondrial fission protein Drp1 and hyperphosphorylated tau in Alzheimer's disease neurons: implications for mitochondrial dysfunction and neuronal damage. Human Molecular Genetics 21:2538-2547.

Marlatt MW, Lucassen PJ, Perry G, Smith MA, Zhu X (2008) ALZHEIMER'S DISEASE: CEREBROVASCULAR DYSFUNCTION, OXIDATIVE STRESS, AND ADVANCED CLINICAL THERAPIES. J Alzheimers Dis 15:199-210.

Mattsson N, Tabatabaei S, Johansson P, Hansson O, Andreasson U, Månsson J-E, Johansson J-O, Olsson B, Wallin A, Svensson J, Blennow K, Zetterberg H (2011) Cerebrospinal Fluid Microglial Markers in Alzheimer's Disease: Elevated Chitotriosidase Activity but Lack of Diagnostic Utility. Neuromolecular medicine 13:151-159.

Maurer I, Zierz S, Möller HJ (2000) A selective defect of cytochrome c oxidase is present in brain of Alzheimer disease patients. Neurobiology of Aging 21:455-462.

Maury E, Ehala-Aleksejev K, Guiot Y, Detry R, Vandenhooft A, Brichard SM (2007) Adipokines oversecreted by omental adipose tissue in human obesity.

McNay EC, Ong CT, McCrimmon RJ, Cresswell J, Bogan JS, Sherwin RS (2010) Hippocampal memory processes are modulated by insulin and high-fat-induced insulin resistance. Neurobiology of Learning and Memory 93:546-553.

McNay EC, Sandusky LA, Pearson-Leary J (2013) Hippocampal Insulin Microinjection and In vivo Microdialysis During Spatial Memory Testing. Journal of Visualized Experiments : JoVE 4451.

McNeilly AD, Williamson R, Sutherland C, Balfour DJK, Stewart CA (2011) High fat feeding promotes simultaneous decline in insulin sensitivity and cognitive performance in a delayed matching and non-matching to position task. Behavioural Brain Research 217:134-141.

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

Milanski M, Degasperi G, Coope A, Morari J, Denis R, Cintra DE, Tsukumo DML, Anhe G, Amaral ME, Takahashi HK, Curi R, Oliveira HC, Carvalheira JBC, Bordin S, Saad MJ, Velloso LA (2009) Saturated Fatty

Acids Produce an Inflammatory Response Predominantly through the Activation of TLR4 Signaling in Hypothalamus: Implications for the Pathogenesis of Obesity. The Journal of Neuroscience 29:359-370.

Miller AA, Spencer SJ (2014) Obesity and neuroinflammation: A pathway to cognitive impairment. Brain, Behavior, and Immunity 42:10-21.

Mody N, Agouni A, McIlroy GD, Platt B, Delibegovic M (2011) Susceptibility to diet-induced obesity and glucose intolerance in the APP SWE/PSEN1 A246E mouse model of Alzheimer's disease is associated with increased brain levels of protein tyrosine phosphatase 1B (PTP1B) and retinol-binding protein 4 (RBP4), and basal phosphorylation of S6 ribosomal protein. Diabetologia 54:2143-2151.

Molteni R, Barnard RJ, Ying Z, Roberts CK, Gómez-Pinilla F (2002) A high-fat, refined sugar diet reduces hippocampal brain-derived neurotrophic factor, neuronal plasticity, and learning. Neuroscience 112:803-814.

Moreira PI, Carvalho C, Zhu X, Smith MA, Perry G (2010) Mitochondrial dysfunction is a trigger of Alzheimer's disease pathophysiology. Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease 1802:2-10.

Morris JK, Honea RA, Vidoni ED, Swerdlow RH, Burns JM (2014) Is Alzheimer's disease a systemic disease? Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease 1842:1340-1349.

Mueller K, Sacher J, Arelin K, Holiga S, Kratzsch J, Villringer A, Schroeter ML (2012) Overweight and obesity are associated with neuronal injury in the human cerebellum and hippocampus in young adults: a combined MRI, serum marker and gene expression study. Transl Psychiatry 2:e200.

Mullan M, Crawford F, Axelman K, Houlden H, Lilius L, Winblad B, Lannfelt L (1992) A pathogenic mutation for probable Alzheimer's disease in the APP gene at the N−terminus of beta−amyloid. Nat Genet 1:345-347.

Mutisya EM, Bowling AC, Beal MF (1994) Cortical Cytochrome Oxidase Activity Is Reduced in Alzheimer's Disease. Journal of Neurochemistry 63:2179-2184.

Naderali EK, Ratcliffe SH, Dale MC (2009) Review: Obesity and Alzheimer's Disease: A Link Between Body Weight and Cognitive Function in Old Age. American Journal of Alzheimer's Disease and Other Dementias 24:445-449.

Nag S (2003) The Blood Brain Barrier. Biology and Research Protocols. Methods in Molecular Medicine 89.

Nakaaki S, Murata Y, Sato J, Shinagawa Y, Hongo J, Tatsumi H, Hirono N, Mimura M, Furukawa TA (2008) Association between apathy/depression and executive function in patients with Alzheimer's disease. International Psychogeriatrics 20:964-975.

Näslund J, Haroutunian V, Mohs R, et al. (2000) Correlation between elevated levels of amyloid β-peptide in the brain and cognitive decline. JAMA 283:1571-1577.

Naville D, Pinteur C, Vega N, Menade Y, Vigier M, Le Bourdais A, Labaronne E, Debard C, Luquain-Costaz C, Bégeot M, Vidal H, Le Magueresse-Battistoni B (2013) Low-dose food contaminants trigger sex-specific, hepatic metabolic changes in the progeny of obese mice. The FASEB Journal 27:3860-3870.

Nedergaard J, Bengtsson T, Cannon B (2007) Unexpected evidence for active brown adipose tissue in adult humans.

Nguyen JCD, Killcross S, Jenkins TA (2014) Obesity and cognitive decline: role of inflammation and vascular changes. Frontiers in Neuroscience 8.

Nickelson KJ, Stromsdorfer KL, Pickering RT, Liu T-W, Ortinau LC, Keating AF, Perfield JW (2012) A Comparison of Inflammatory and Oxidative Stress Markers in Adipose Tissue from Weight-Matched Obese Male and Female Mice. Experimental Diabetes Research 2012:8.

Ninomiya T (2014) Diabetes Mellitus and Dementia. Curr Diab Rep 14:1-9.

Niwa K, Carlson GA, Iadecola C (2000) Exogenous Abeta1-40 Reproduces Cerebrovascular Alterations Resulting From Amyloid Precursor Protein Overexpression in Mice. J Cereb Blood Flow Metab 20:1659-1668.

Okello A, Edison P, Archer HA, Turkheimer FE, Kennedy J, Bullock R, Walker Z, Kennedy A, Fox N, Rossor M, Brooks DJ (2009a) Microglial activation and amyloid deposition in mild cognitive impairment: A PET study. Neurology 72:56-62.

Okello A, Koivunen J, Edison P, Archer HA, Turkheimer FE, Någren K, Bullock R, Walker Z, Kennedy A, Fox NC, Rossor MN, Rinne JO, Brooks DJ (2009b) Conversion of amyloid positive and negative MCI to AD over 3 years: An (11)C-PIB PET study. Neurology 73:754-760.

Oksman M, Iivonen H, Hogyes E, Amtul Z, Penke B, Leenders I, Broersen L, Lütjohann D, Hartmann T, Tanila H (2006) Impact of different saturated fatty acid, polyunsaturated fatty acid and cholesterol containing diets on beta-amyloid accumulation in APP/PS1 transgenic mice. Neurobiology of Disease 23:563-572.

Oliveros LB, Videla AM, Giménez MS (2004) Effect of dietary fat saturation on lipid metabolism, arachidonic acid turnover and peritoneal macrophage oxidative stress in mice. Brazilian Journal of Medical and Biological Research 37:311-320.

Olson MI, Shaw CM (1969) Presenile dementia and Alzheimer's disease in mongolism. Brain 92:147-156.

Ott A, Breteler MMB, Harskamp Fv, Stijnen T, Hofman A (1998) Incidence and Risk of Dementia: The Rotterdam study. American Journal of Epidemiology 147:574-580.

Ott A, Stolk RP, Van Harskamp F, Pols HAP, Hofman A, Breteler MMB (1999) Diabetes mellitus and the risk of dementia: The Rotterdam Study. Neurology 53:1937-1942.

Ouyang S, Hsuchou H, Kastin AJ, Wang Y, Yu C, Pan W (2014) Diet-induced obesity suppresses expression of many proteins at the blood-brain barrier. J Cereb Blood Flow Metab 34:43-51.

Pannacciulli N, Del Parigi A, Chen K, Le DSNT, Reiman EM, Tataranni PA (2006) Brain abnormalities in human obesity: A voxel-based morphometric study. NeuroImage 31:1419-1425.

Park CR (2001) Cognitive effects of insulin in the central nervous system. Neuroscience & Biobehavioral Reviews 25:311-323.

Parra V, Verdejo HE, Iglewski M, del Campo A, Troncoso R, Jones D, Zhu Y, Kuzmicic J, Pennanen C, Lopez-Crisosto C, Jaña F, Ferreira J, Noguera E, Chiong M, Bernlohr DA, Klip A, Hill JA, Rothermel BA, Abel ED, Zorzano A, Lavandero S (2014) Insulin Stimulates Mitochondrial Fusion and Function in Cardiomyocytes via the Akt-mTOR-NFκB-Opa-1 Signaling Pathway. Diabetes 63:75-88.

Paul J, Strickland S, Melchor JP (2007) Fibrin deposition accelerates neurovascular damage and neuroinflammation in mouse models of Alzheimer's disease. The Journal of Experimental Medicine 204:1999-2008.

Peila R, Rodriguez BL, Launer LJ (2002) Type 2 Diabetes, APOE Gene, and the Risk for Dementia and Related Pathologies: The Honolulu-Asia Aging Study. Diabetes 51:1256-1262.

Pepping JK, Freeman LR, Gupta S, Keller JN, Bruce-Keller AJ (2013) NOX2 deficiency attenuates markers of adiposopathy and brain injury induced by high-fat diet.

Perry VH, Holmes C (2014) Microglial priming in neurodegenerative disease. Nat Rev Neurol 10:217-224.

Perry VH, Nicoll JAR, Holmes C (2010) Microglia in neurodegenerative disease. Nat Rev Neurol 6:193-201.

Perry VH, Teeling J (2013) Microglia and macrophages of the central nervous system: the contribution of microglia priming and systemic inflammation to chronic neurodegeneration. Semin Immunopathol 35:601-612.

Persidsky Y, Ramirez S, Haorah J, Kanmogne G (2006) Blood–brain Barrier: Structural Components and Function Under Physiologic and Pathologic Conditions. Jrnl Neuroimmune Pharm 1:223-236.

Pettersson US, Waldén TB, Carlsson P-O, Jansson L, Phillipson M (2012) Female Mice are Protected against High-Fat Diet Induced Metabolic Syndrome and Increase the Regulatory T Cell Population in Adipose Tissue. PLoS ONE 7:e46057.

Pistell PJ, Morrison CD, Gupta S, Knight AG, Keller JN, Ingram DK, Bruce-Keller AJ (2010) Cognitive impairment following high fat diet consumption is associated with brain inflammation. Journal of Neuroimmunology 219:25-32.

Posey KA, Clegg DJ, Printz RL, Byun J, Morton GJ, Vivekanandan-Giri A, Pennathur S, Baskin DG, Heinecke JW, Woods SC, Schwartz MW, Niswender KD (2009) Hypothalamic proinflammatory lipid accumulation, inflammation, and insulin resistance in rats fed a high-fat diet.

Power ML, Schulkin J (2008) Sex differences in fat storage, fat metabolism, and the health risks from obesity: possible evolutionary origins. British Journal of Nutrition 99:931-940.

Profenno LA, Porsteinsson AP, Faraone SV (2010) Meta-Analysis of Alzheimer's Disease Risk with Obesity, Diabetes, and Related Disorders. Biological Psychiatry 67:505-512.

Puig KL, Floden AM, Adhikari R, Golovko MY, Combs CK (2012) Amyloid Precursor Protein and Proinflammatory Changes Are Regulated in Brain and Adipose Tissue in a Murine Model of High Fat Diet-Induced Obesity. PLoS ONE 7:e30378.

Qatanani M, Lazar MA (2007) Mechanisms of obesity-associated insulin resistance: many choices on the menu. Genes & Development 21:1443-1455.

Qin, H., A. T. Holdbrooks, et al. (2012). "SOCS3 Deficiency Promotes M1 Macrophage Polarization and Inflammation." Journal of immunology (Baltimore, Md. : 1950) 189(7): 3439-3448.

Qiu C, Kivipelto M, von Strauss E (2009) Epidemiology of Alzheimer's disease: occurrence, determinants, and strategies toward intervention. Dialogues in Clinical Neuroscience 11:111-128.

Qiu C, Winblad B, Fratiglioni L (2005) The age-dependent relation of blood pressure to cognitive function and dementia. The Lancet Neurology 4:487-499.

Querfurth HW, LaFerla FM (2010) Alzheimer's Disease. New England Journal of Medicine 362:329-344.

Raji CA, Ho AJ, Parikshak NN, Becker JT, Lopez OL, Kuller LH, Hua X, Leow AD, Toga AW, Thompson PM (2010) Brain structure and obesity. Human Brain Mapping 31:353-364.

Reddy PH (2009) Amyloid beta, mitochondrial structural and functional dynamics in Alzheimer's disease. Exp Neurol 218:286-292.

Reddy PH, Beal MF (2008) Amyloid beta, mitochondrial dysfunction and synaptic damage: implications for cognitive decline in aging and Alzheimer's disease. Trends in Molecular Medicine 14:45-53.

Reddy PH, Manczak M, Mao P, Calkins MJ, Reddy AP, Shirendeb U (2010) Amyloid-β and Mitochondria in Aging and Alzheimer's Disease: Implications for Synaptic Damage and Cognitive Decline. J Alzheimers Dis 20:S499-S512.

Reddy PH, McWeeney S, Park BS, Manczak M, Gutala RV, Partovi D, Jung Y, Yau V, Searles R, Mori M, Quinn J (2004) Gene expression profiles of transcripts in amyloid precursor protein transgenic mice: up-regulation of mitochondrial metabolism and apoptotic genes is an early cellular change in Alzheimer's disease. Human Molecular Genetics 13:1225-1240.

Redinger RN (2009) Fat storage and the biology of energy expenditure. Translational Research 154:52-60.

Refolo LM, Pappolla MA, Malester B, LaFrancois J, Bryant-Thomas T, Wang R, Tint GS, Sambamurti K, Duff K (2000) Hypercholesterolemia Accelerates the Alzheimer's Amyloid Pathology in a Transgenic Mouse Model. Neurobiology of Disease 7:321-331.

Reichelt AC, Maniam J, Westbrook RF, Morris MJ (2015) Dietary-induced obesity disrupts trace fear conditioning and decreases hippocampal reelin expression. Brain, Behavior, and Immunity 43:68-75.

Reitz C, Mayeux R (2014) Alzheimer disease: Epidemiology, diagnostic criteria, risk factors and biomarkers. Biochemical Pharmacology 88:640-651.

Rhein V, Eckert A (2007) Effects of Alzheimer's amyloid-beta and tau protein on mitochondrial function—role of glucose metabolism and insulin signalling. Archives Of Physiology And Biochemistry 113:131-141.

Rivera EJ, Goldin A, Fulmer N, Tavares R, Wands JR, De La Monte SM (2005) Insulin and insulin-like growth factor expression and function deteriorate with progression of Alzheimer's disease: Link to brain reductions in acetylcholine. Journal of Alzheimer's Disease 8:247-268.

Rosengren A, Skoog I, Gustafson D, Wilhelmsen L (2005) BOdy mass index, other cardiovascular risk factors, and hospitalization for dementia. Archives of Internal Medicine 165:321-326.

Rovelet-Lecrux A, Hannequin D, Raux G, Le Meur N, Laquerrière A, Vital A, Dumanchin C, Feuillette S, Brice A, Vercelletto M, Dubas F, Frebourg T, Campion D (2006) APP locus duplication causes autosomal dominant early-onset Alzheimer disease with cerebral amyloid angiopathy. Nat Genet 38:24-26.

Saely CH, Geiger K, Drexel H (2012) Brown versus White Adipose Tissue: A Mini-Review. Gerontology 58:15-23.

Santos RX, Correia SC, Alves MG, Oliveira PF, Cardoso S, Carvalho C, Duarte AI, Santos MS, Moreira PI (2014) Insulin therapy modulates mitochondrial dynamics and biogenesis, autophagy and tau protein phosphorylation in the brain of type 1 diabetic rats. Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease 1842:1154-1166.

Scheuner D, Eckman C, Jensen M, Song X, Citron M, Suzuki N, Bird TD, Hardy J, Hutton M, Kukull W, Larson E, Levy-Lahad L, Viitanen M, Peskind E, Poorkaj P, Schellenberg G, Tanzi R, W. Wasco, L. Lannfelt, Selkoe D, Younkin S (1996) Secreted amyloid bold beta−protein similar to that in the senile plaques of Alzheimer's disease is increased in vivo by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease. Nature Medicine 2:864-870.

Selkoe DJ (1991) The molecular pathology of Alzheimer's disease. Neuron 6:487-498.

Selkoe DJ (2001) Alzheimer's Disease: Genes, Proteins, and Therapy. Physiological Reviews 81:741-766.

Sellbom KS, Gunstad J (2012) Cognitive function and decline in obesity. Journal of Alzheimer's Disease 30:S89-S95.

Senthil Kumar SPD, Shen M, Spicer EG, Goudjo-Ako AJ, Stumph JD, Zhang J, Shi H (2014) Distinct metabolic effects following short-term exposure of different high-fat diets in male and female mice. Endocrine Journal 61:457-470.

Sepulveda FJ, Parodi J, Peoples RW, Opazo C, Aguayo LG (2010) Synaptotoxicity of Alzheimer Beta Amyloid Can Be Explained by Its Membrane Perforating Property. PLoS ONE 5:e11820.

Serrano F, Klann E (2004) Reactive oxygen species and synaptic plasticity in the aging hippocampus. Ageing Research Reviews 3:431-443.

Shanik MH, Xu Y, Škrha J, Dankner R, Zick Y, Roth J (2008) Insulin Resistance and Hyperinsulinemia: Is hyperinsulinemia the cart or the horse? Diabetes Care 31:S262-S268.

Shankar G, Walsh D (2009) Alzheimer's disease: synaptic dysfunction and Abeta. Molecular Neurodegeneration 4:48.

Sharma S, Taliyan R (2014) Neuroprotective role of Indirubin-3’-monoxime, a GSKβ inhibitor in high fat diet induced cognitive impairment in mice. Biochemical and Biophysical Research Communications 452:1009-1015.

Sharma S, Taliyan R, Ramagiri S (2014) Histone Deacetylase Inhibitor, Trichostatin A, Improves Learning and Memory in High-Fat Diet-Induced Cognitive Deficits in Mice. Journal of Molecular Neuroscience 1-11.

Sherrington R, Rogaev EI, Liang Y, Rogaeva EA, Levesque G, Ikeda M, Chi H, Lin C, Li G, Holman K, Tsuda T, Mar L, Foncin JF, Bruni AC, Montesi MP, Sorbi S, Rainero I, Pinessi L, Nee L, Chumakov I, Pollen D, Brookes A, Sanseau P, Polinsky RJ, Wasco W, Da Silva HAR, Haines JL, Pericak-Vance MA, Tanzi RE, Roses AD, Fraser PE, Rommens JM, St George-Hyslop PH (1995) Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. Nature 375:754-760.

Shie F-S, Jin L-W, Cook DG, Leverenz JB, LeBoeuf RC (2002) Diet-induced hypercholesterolemia enhances brain Aβ accumulation in transgenic mice. NeuroReport 13:455-459.

Shu CJ, Benoist C, Mathis D (2012) The immune system's involvement in obesity-driven type 2 diabetes. Seminars in Immunology 24:436-442.

Sims-Robinson C, Kim B, Rosko A, Feldman EL (2010) How does diabetes accelerate Alzheimer disease pathology? Nat Rev Neurol 6:551-559.

Sinforiani E, Citterio A, Zucchella C, Bono G, Corbetta S, Merlo P, Mauri M (2010) Impact of Gender Differences on the Outcome of Alzheimer's Disease. Dementia and Geriatric Cognitive Disorders 30:147-154.

Skeberdis VA, Lan J-y, Zheng X, Zukin RS, Bennett MVL (2001) Insulin promotes rapid delivery of N-methyl-d-aspartate receptors to the cell surface by exocytosis. Proceedings of the National Academy of Sciences 98:3561-3566.

Skurk T, Alberti-Huber C, Herder C, Hauner H (2007) Relationship between Adipocyte Size and Adipokine Expression and Secretion. The Journal of Clinical Endocrinology & Metabolism 92:1023-1033.

Small DH, Gasperini R, Vincent AJ, Hung AC, Foa L (2009) The Role of Aβ-Induced Calcium Dysregulation in the Pathogenesis of Alzheimer's Disease. Journal of Alzheimer's Disease 16:225-233.

Sobesky JL, Barrientos RM, De May HS, Thompson BM, Weber MD, Watkins LR, Maier SF (2014) High-fat diet consumption disrupts memory and primes elevations in hippocampal IL-1β, an effect that can be prevented with dietary reversal or IL-1 receptor antagonism. Brain, Behavior, and Immunity 42:22-32.

Son SM, Shin HJ, Mook-Jung I (2011) Insulin Resistance and Alzheimer's Disease.

Souza CTD, Araujo EP, Bordin S, Ashimine R, Zollner RL, Boschero AC, Saad MJA, Velloso LA (2005) Consumption of a Fat-Rich Diet Activates a Proinflammatory Response and Induces Insulin Resistance in the Hypothalamus. Endocrinology 146:4192-4199.

Spalding KL, Arner E, Westermark PO, Bernard S, Buchholz BA, Bergmann O, Blomqvist L, Hoffstedt J, Naslund E, Britton T, Concha H, Hassan M, Ryden M, Frisen J, Arner P (2008) Dynamics of fat cell turnover in humans. Nature 453:783-787.

Sperling RA, Dickerson BC, Pihlajamaki M, Vannini P, LaViolette PS, Vitolo OV, Hedden T, Becker JA, Rentz DM, Selkoe DJ, Johnson KA (2010) Functional Alterations in Memory Networks in Early Alzheimer's Disease. Neuromolecular medicine 12:27-43.

Spielman LJ, Little JP, Klegeris A (2014) Inflammation and insulin/IGF-1 resistance as the possible link between obesity and neurodegeneration. Journal of Neuroimmunology 273:8-21.

Steen E, Terry BM, Rivera EJ, Cannon JL, Neely TR, Tavares R, Xu XJ, Wands JR, De La Monte SM (2005) Impaired insulin and insulin-like growth factor expression and signaling mechanisms in Alzheimer's disease - Is this type 3 diabetes? Journal of Alzheimer's Disease 7:63-80.

Stevens LM, Brown RE (2015) Reference and working memory deficits in the 3xTg-AD mouse between 2 and 15-months of age: A cross-sectional study. Behavioural Brain Research 278:496-505.

Stewart R, Liolitsa D (1999) Type 2 diabetes mellitus, cognitive impairment and dementia. Diabetic Medicine 16:93-112.

Stewart R, Masaki K, Xue Q, et al. (2005) A 32-year prospective study of change in body weight and incident dementia: The honolulu-asia aging study. Archives of Neurology 62:55-60.

Stockhorst U, de Fries D, Steingrueber H-J, Scherbaum WA (2004) Insulin and the CNS: effects on food intake, memory, and endocrine parameters and the role of intranasal insulin administration in humans. Physiology & Behavior 83:47-54.

Strachan MWJ, Deary IJ, Ewing FME, Frier BM (1997) Is Type II Diabetes Associated With an Increased Risk of Cognitive Dysfunction?: A critical review of published studies. Diabetes Care 20:438-445.

Stranahan AM, Mattson MP (2012) Recruiting adaptive cellular stress responses for successful brain ageing. Nat Rev Neurosci 13:209-216.

Stranahan AM, Norman ED, Lee K, Cutler RG, Telljohann RS, Egan JM, Mattson MP (2008) Diet-induced insulin resistance impairs hippocampal synaptic plasticity and cognition in middle-aged rats. Hippocampus 18:1085-1088.

Strissel KJ, DeFuria J, Shaul ME, Bennett G, Greenberg AS, Obin MS (2010) T Cell Recruitment and Th1 Polarization in Adipose Tissue During Diet-Induced Obesity in C57BL/6 mice. Obesity (Silver Spring, Md) 18:1918-1925.

Strissel KJ, Stancheva Z, Miyoshi H, Perfield JW, DeFuria J, Jick Z, Greenberg AS, Obin MS (2007) Adipocyte Death, Adipose Tissue Remodeling, and Obesity Complications. Diabetes 56:2910-2918.

Strittmatter WJ, Saunders AM, Schmechel D, Pericak-Vance M, Enghild J, Salvesen GS, Roses AD (1993) Apolipoprotein E: high-avidity binding to beta-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. Proceedings of the National Academy of Sciences of the United States of America 90:1977-1981.

Studzinski CM, Li F, Bruce-Keller AJ, Fernandez-Kim SO, Le Z, Weidner AM, Markesbery WR, Murphy MP, Keller JN (2009) Effects of short-term Western diet on cerebral oxidative stress and diabetes related factors in APP × PS1 knock-in mice. Journal of Neurochemistry 108:860-866.

Stutzmann GE, Mattson MP (2011) Endoplasmic Reticulum Ca(2+) Handling in Excitable Cells in Health and Disease. Pharmacological Reviews 63:700-727.

Swan GE, DeCarli C, Miller BL, Reed T, Wolf PA, Jack LM, Carmelli D (1998) Association of midlife blood pressure to late-life cognitive decline and brain morphology. Neurology 51:986-993.

Sweat V, Starr V, Bruehl H, Arentoft A, Tirsi A, Javier E, Convit A (2008) C-Reactive Protein is Linked to Lower Cognitive Performance in Overweight and Obese Women. Inflammation 31:198-207.

Swerdlow RH, Burns JM, Khan SM (2010) The Alzheimer's Disease Mitochondrial Cascade Hypothesis. Journal of Alzheimer's Disease 20:265-279.

Swerdlow RH, Khan SM (2004) A “mitochondrial cascade hypothesis” for sporadic Alzheimer's disease. Medical Hypotheses 63:8-20.

Swerdlow RH, Khan SM (2009) The Alzheimer's disease mitochondrial cascade hypothesis: An update. Exp Neurol 218:308-315.

Szabados T, Dul C, Majtényi K, Hargitai J, Pénzes Z, Urbanics R (2004) A chronic Alzheimer's model evoked by mitochondrial poison sodium azide for pharmacological investigations. Behavioural Brain Research 154:31-40.

Takeda S, Sato N, Morishita R (2014) Systemic inflammation, blood-brain barrier vulnerability and cognitive / non-cognitive symptoms in Alzheimer disease: Relevance to pathogenesis and therapy. Frontiers in Aging Neuroscience 6.

Taki Y, Kinomura S, Sato K, Inoue K, Goto R, Okada K, Uchida S, Kawashima R, Fukuda H (2008) Relationship Between Body Mass Index and Gray Matter Volume in 1,428 Healthy Individuals. Obesity 16:119-124.

Talbot K, Wang H-Y, Kazi H, Han L-Y, Bakshi KP, Stucky A, Fuino RL, Kawaguchi KR, Samoyedny AJ, Wilson RS, Arvanitakis Z, Schneider JA, Wolf BA, Bennett DA, Trojanowski JQ, Arnold SE (2012) Demonstrated brain insulin resistance in Alzheimer's disease patients is associated with IGF-1 resistance, IRS-1 dysregulation, and cognitive decline. The Journal of Clinical Investigation 122:1316-1338.

Terry RD, Masliah E, Salmon DP, Butters N, DeTeresa R, Hill R, Hansen LA, Katzman R (1991) Physical basis of cognitive alterations in Alzheimer's disease: synapse loss is the major correlate of cognitive impairment. Ann Neurology 30:572-580.

Terwel, D., R. Lasrado, et al. (2005). "Changed Conformation of Mutant Tau-P301L Underlies the Moribund Tauopathy, Absent in Progressive, Nonlethal Axonopathy of Tau-4R/2N Transgenic Mice." Journal of Biological Chemistry 280(5): 3963-3973.

Tezapsidis N, Johnston JM, Smith MA, Ashford JW, Casadesus G, Robakis NK, Wolozin B, Perry G, Zhu X, Greco SJ, Sarkar S (2009) Leptin: A Novel Therapeutic Strategy for Alzheimer's Disease. J Alzheimers Dis 16:731-740.

Thaler JP, Yi C-X, Schur EA, Guyenet SJ, Hwang BH, Dietrich MO, Zhao X, Sarruf DA, Izgur V, Maravilla KR, Nguyen HT, Fischer JD, Matsen ME, Wisse BE, Morton GJ, Horvath TL, Baskin DG, Tsch, xF, p MH, Schwartz MW (2012) Obesity is associated with hypothalamic injury in rodents and humans. The Journal of Clinical Investigation 122:153-162.

Thinakaran, G. and A. T. Parent (2004). "Identification of the role of presenilins beyond Alzheimer's disease." Pharmacological Research 50(4): 411-418.

Thirumangalakudi L, Prakasam A, Zhang R, Bimonte-Nelson H, Sambamurti K, Kindy MS, Bhat NR (2008) High cholesterol-induced neuroinflammation and amyloid precursor protein processing correlate with loss of working memory in mice. Journal of Neurochemistry 106:475-485.

Tolppanen A-M, Ngandu T, Kåreholt I, Laatikainen T, Rusanen M, Soininen H, Kivipelto M (2014) Midlife and Late-Life Body Mass Index and Late-Life Dementia: Results from a Prospective Population-Based Cohort. Journal of Alzheimer's Disease 38:201-209.

Tucsek Z, Toth P, Sosnowska D, Gautam T, Mitschelen M, Koller A, Szalai G, Sonntag WE, Ungvari Z, Csiszar A (2014a) Obesity in Aging Exacerbates Blood–Brain Barrier Disruption, Neuroinflammation, and Oxidative Stress in the Mouse Hippocampus: Effects on Expression of Genes Involved in Beta-Amyloid Generation and Alzheimer's Disease. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences 69:1212-1226.

Tucsek Z, Toth P, Tarantini S, Sosnowska D, Gautam T, Warrington JP, Giles CB, Wren JD, Koller A, Ballabh P, Sonntag WE, Ungvari Z, Csiszar A (2014b) Aging Exacerbates Obesity-induced Cerebromicrovascular Rarefaction, Neurovascular Uncoupling, and Cognitive Decline in Mice. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences.

Ujiie M, Dickstein DL, Carlow DA, Jefferies WA (2003) Blood—Brain Barrier Permeability Precedes Senile Plaque Formation in an Alzheimer Disease Model. Microcirculation 10:463-470.

Umegaki H (2012) Neurodegeneration in Diabetes Mellitus. In: Neurodegenerative Diseases, vol. 724 (Ahmad, S., ed), pp 258-265: Springer US.

Usman S, Chaudhary HR, Asif A, Yahya MI (2010) Severity and Risk Factors of Depression in Alzheimer's Disease. Journal of the College of Physicians and Surgeons Pakistan 20:327-330.

Valladolid-Acebes I, Fole A, Martín M, Morales L, Victoria Cano M, Ruiz-Gayo M, Olmo ND (2013) Spatial memory impairment and changes in hippocampal morphology are triggered by high-fat diets in adolescent mice. Is there a role of leptin? Neurobiology of Learning and Memory 106:18-25.

Valladolid-Acebes I, Stucchi P, Cano V, Fernández-Alfonso MS, Merino B, Gil-Ortega M, Fole A, Morales L, Ruiz-Gayo M, Olmo ND (2011) High-fat diets impair spatial learning in the radial-arm maze in mice. Neurobiology of Learning and Memory 95:80-85.

van der Heide LP, Ramakers GMJ, Smidt MP (2006) Insulin signaling in the central nervous system: Learning to survive. Progress in Neurobiology 79:205-221.

Van der Mussele S, Bekelaar K, Le Bastard N, Vermeiren Y, Saerens J, Somers N, Mariën P, Goeman J, De Deyn PP, Engelborghs S (2013) Prevalence and associated behavioral symptoms of depression in mild cognitive impairment and dementia due to Alzheimer's disease. International Journal of Geriatric Psychiatry 28:947-958.

Vandal M, White PJ, Tremblay C, St-Amour I, Chevrier G, Emond V, Lefrançois D, Virgili J, Planel E, Giguere Y, Marette A, Calon F (2014) Insulin Reverses the High-Fat Diet–Induced Increase in Brain Aβ and Improves Memory in an Animal Model of Alzheimer Disease. Diabetes 63:4291-4301.

Verdier Y, B. P (2004) Binding sites of amyloid beta-peptide in cell plasma membrane and implications for Alzheimer's disease. Curr Protein Pept Sci 5:19-31.

Viña J, Lloret A (2010) Why women have more Alzheimer's disease than men: Gender and mitochondrial toxicity of amyloid-β peptide. Journal of Alzheimer's Disease 20:S527-S533.

Waldstein SR, Katzel LI (2005) Interactive relations of central versus total obesity and blood pressure to cognitive function. Int J Obes Relat Metab Disord 30:201-207.

Wallace DC, Stugard C, Murdock D, Schurr T, Brown MD (1997) Ancient mtDNA sequences in the human nuclear genome: A potential source of errors in identifying pathogenic mutations. Proceedings of the National Academy of Sciences 94:14900-14905.

Walsh DM, Hartley DM, Kusumoto Y, Fezoui Y, Condron MM, Lomakin A, Benedek GB, Selkoe DJ, Teplow DB (1999) Amyloid β-Protein Fibrillogenesis: STRUCTURE AND BIOLOGICAL ACTIVITY OF PROTOFIBRILLAR INTERMEDIATES. Journal of Biological Chemistry 274:25945-25952.

Walsh DM, Klyubin I, Fadeeva JV, Cullen WK, Anwyl R, Wolfe MS, Rowan MJ, Selkoe DJ (2002) Naturally secreted oligomers of amyloid [beta] protein potently inhibit hippocampal long-term potentiation in vivo. Nature 416:535-539.

Walsh DM, Selkoe DJ (2004) Deciphering the Molecular Basis of Memory Failure in Alzheimer's Disease. Neuron 44:181-193.

Wan Q, Xiong ZG, Man HY, Ackerley CA, Braunton J, Lu WY, Becker LE, MacDonald JF, Wang YT (1997) Recruitment of functional GABA(A) receptors to postsynaptic domains by insulin. Nature 388:686-690.

Wang J, Xiong S, Xie C, Markesbery WR, Lovell MA (2005) Increased oxidative damage in nuclear and mitochondrial DNA in Alzheimer's disease. Journal of Neurochemistry 93:953-962.

Wang Q, Walsh DM, Rowan MJ, Selkoe DJ, Anwyl R (2004) Block of Long-Term Potentiation by Naturally Secreted and Synthetic Amyloid β-Peptide in Hippocampal Slices Is Mediated via Activation of the Kinases c-Jun N-Terminal Kinase, Cyclin-Dependent Kinase 5, and p38 Mitogen-Activated Protein Kinase as well as Metabotropic Glutamate Receptor Type 5. The Journal of Neuroscience 24:3370-3378.

Wang X, Su B, Fujioka H, Zhu X (2008) Dynamin-Like Protein 1 Reduction Underlies Mitochondrial Morphology and Distribution Abnormalities in Fibroblasts from Sporadic Alzheimer's Disease Patients. The American Journal of Pathology 173:470-482.

Wang X, Su B, Lee H-g, Li X, Perry G, Smith MA, Zhu X (2009) Impaired Balance of Mitochondrial Fission and Fusion in Alzheimer's Disease. The Journal of Neuroscience 29:9090-9103.

Wang X, Wang W, Li L, Perry G, Lee H-g, Zhu X (2014) Oxidative stress and mitochondrial dysfunction in Alzheimer's disease. Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease 1842:1240-1247.

Ward M, Carlsson C, Trivedi M, Sager M, Johnson S (2005) The effect of body mass index on global brain volume in middle-aged adults: a cross sectional study. BMC Neurology 5:23.

Watson GS, Craft S (2004) Modulation of memory by insulin and glucose: neuropsychological observations in Alzheimer's disease. European Journal of Pharmacology 490:97-113.

Wattmo C, Wallin A, Londos E, Minthon L (2011) Predictors of long-term cognitive outcome in Alzheimer's disease. Alzheimer's Research & Therapy 3:23.

Webster MT, Pearce BR, Bowen DM, Francis PT (1998) The effects of perturbed energy metabolism on the processing of amyloid precursor protein in PC12 cells. J Neural Transm 105:839-853.

Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW (2003) Obesity is associated with macrophage accumulation in adipose tissue. Journal of Clinical Investigation 112:1796-1808.

Wellen KE, Hotamisligil GS (2005) Inflammation, stress, and diabetes. Journal of Clinical Investigation 115:1111-1119.

Whitmer RA, Gunderson EP, Barrett-Connor E, Quesenberry CP, Yaffe K (2005a) Obesity in middle age and future risk of dementia: a 27 year longitudinal population based study.

Whitmer RA, Gustafson DR, Barrett-Connor E, Haan MN, Gunderson EP, Yaffe K (2008) Central obesity and increased risk of dementia more than three decades later. Neurology 71:1057-1064.

Whitmer RA, Sidney S, Selby J, Johnston SC, Yaffe K (2005b) Midlife cardiovascular risk factors and risk of dementia in late life. Neurology 64:277-281.

WHO (2014) Obesity and overweight. http://wwwwhoint/mediacentre/factsheets/fs311/en/.

Wichmann MA, Cruickshanks KJ, Carlsson CM, Chappell R, Fischer ME, Klein BEK, Klein R, Tsai MY, Schubert CR (2014) Long-Term Systemic Inflammation and Cognitive Impairment in a Population-Based Cohort. Journal of the American Geriatrics Society 62:1683-1691.

Winocur G, Greenwood CE (2005) Studies of the effects of high fat diets on cognitive function in a rat model. Neurobiology of Aging 26:46-49.

Wisniewski T, Ghiso J, Frangione B (1991) Peptides homologous to the amyloid protein of Alzheimer's disease containing a glutamine for glutamic acid substitution have accelerated amyloid fibril formation. Biochemical and Biophysical Research Communications 179:1247-1254.

Wolburg H, Lippoldt A (2002) Tight junctions of the blood–brain barrier: development, composition and regulation. Vascular Pharmacology 38:323-337.

Wolf PA, Beiser A, Elias MF, Au R, Vasan RS, Seshadri S (2007) Relation of Obesity to Cognitive Function: Importance of Central Obesity and Synergistic Influence of Concomitant Hypertension. The Framingham Heart Study. Current Alzheimer Research 4:111-116.

Wu H-Y, Hudry E, Hashimoto T, Kuchibhotla K, Rozkalne A, Fan Z, Spires-Jones T, Xie H, Arbel-Ornath M, Grosskreutz CL, Bacskai BJ, Hyman BT (2010) Amyloid Beta (Aβ) Induces the Morphological Neurodegenerative Triad of Spine Loss, Dendritic Simplification, and Neuritic Dystrophies through Calcineurin (CaN) Activation. J Neurosci 30:2636-2649.

Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, Sole J, Nichols A, Ross JS, Tartaglia LA, Chen H (2003) Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. The Journal of Clinical Investigation 112:1821-1830.

Xu WL, Atti AR, Gatz M, Pedersen NL, Johansson B, Fratiglioni L (2011) Midlife overweight and obesity increase late-life dementia risk: a population-based twin study. . Neurology 76::1568–1574.

Yan SD, Chen X, Fu J, Chen M, Zhu H, Roher A, Slattery T, Zhao L, Nagashima M, Morser J, Migheli A, Nawroth P, Stern D, Schmidt AM (1996) RAGE and amyloid-[beta] peptide neurotoxicity in Alzheimer's disease. Nature 382:685-691.

Yang Y, Smith DL, Keating KD, Allison DB, Nagy TR (2014) Variations in body weight, food intake, and body composition after long-term high-fat diet feeding in C57BL/6J mice. Obesity n/a-n/a.

Yao J, Irwin RW, Zhao L, Nilsen J, Hamilton RT, Brinton RD (2009) Mitochondrial bioenergetic deficit precedes Alzheimer's pathology in female mouse model of Alzheimer's disease. Proceedings of the National Academy of Sciences of the United States of America 106:14670-14675.

Yu H, Bi Y, Ma W, He L, Yuan L, Feng J, Xiao R (2010) Long-term effects of high lipid and high energy diet on serum lipid, brain fatty acid composition, and memory and learning ability in mice. International Journal of Developmental Neuroscience 28:271-276.

Zhao W-Q, Alkon DL (2001) Role of insulin and insulin receptor in learning and memory. Molecular and Cellular Endocrinology 177:125-134.

Zhao W-Q, Chen H, Quon MJ, Alkon DL (2004) Insulin and the insulin receptor in experimental models of learning and memory. European Journal of Pharmacology 490:71-81.

Zhao W, Chen H, Xu H, Moore E, Meiri N, Quon MJ, Alkon DL (1999) Brain Insulin Receptors and Spatial Memory: CORRELATED CHANGES IN GENE EXPRESSION, TYROSINE PHOSPHORYLATION, AND SIGNALING

MOLECULES IN THE HIPPOCAMPUS OF WATER MAZE TRAINED RATS. Journal of Biological Chemistry 274:34893-34902.

Zlokovic BV (2011) Neurovascular pathways to neurodegeneration in Alzheimer's disease and other disorders. Nat Rev Neurosci 12:723-738.

Zlokovic BV, Deane R, Sagare AP, Bell RD, Winkler EA (2010) Low-density lipoprotein receptor-related protein-1 : a serial clearance homeostatic mechanism controlling Alzheimer's amyloid β-peptide elimination from the brain. Journal of Neurochemistry 115:1077-1089.

Zuliani G, Cavalieri M, Galvani M, Passaro A, Munari MR, Bosi C, Zurlo A, Fellin R (2008) Markers of endothelial dysfunction in older subjects with late onset Alzheimer's disease or vascular dementia. Journal of the Neurological Sciences 272:164-170.