AGE-RELATED WEIGHT LOSS IN THE 5XFAD MOUSE MODEL OF ALZHEIMER'S DISEASE: A BEHAVIOURAL AND HORMONAL ANALYSIS

by

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DEDICATION PAGE

I would like to dedicate this work to all of those who have committed their time to understanding the mechanisms behind Alzheimer's disease. Without these people little would be known about this disease that afflicts so many. I would also like to dedicate this work to Dr. Brown and the rest of the Brown lab for supporting this work and spending years of their lives to understand this disease. I would also like to dedicate this work to Dr. Anini for his support in teaching me the essentials of molecular analysis. Finally Thanks to Stephanie Pelletier and Mike Landsman for enduring my horrid humour and spending hours with me running experiments.

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ABSTRACT

The 5xFAD mouse is a double transgenic model of Alzheimer's disease (AD), which carries an amyloid precursor protein (APP) transgene with three mutations, and a presenilin-1 transgene with two mutations. Beta amyloid (Aβ)-plaques emerge at two months of age in this mouse model and age-related weight loss begins at 9 months of age. This thesis investigated age-related changes in body weight, feeding behaviour, activity levels, feeding-related hormones, and leptin, uncoupling protein-1, uncoupling protein-2, and hormone sensitive lipase mRNA levels in female 5xFAD mice and their wild-type (WT; C57BL/6JxSJL/J F1) controls from 3 to 12 months of age. We found that 5xFAD mice were hypoactive in climbing and rearing ($p<0.05$), and spent more time remaining still ($p<0.05$). Differences in food intake and body weight were shown between 9 and 12 months of age, where WT ate more ($p<0.05$) and weighed more than 5xFAD mice ($p<0.05$). At 12 months of age 5xFAD mice had less perigonadal white adipose tissue (WAT) than WT mice ($p<0.05$), but there were no genotype differences in interscapular brown adipose tissue (BAT) mass, or in muscle mass. We collected blood at 12 months of age and measured the plasma concentration of insulin and glucose, which did not differ between genotypes ($p>0.05$). Expression of leptin mRNA, UCP-2, UCP-1, and hormone sensitive lipase were measured in WAT. The 5xFAD expressed reduced mRNA levels of leptin, UCP-2, and hormone sensitive lipase ($p<0.05$). Transgenic mice expressed more UCP-1 than WT controls ($p<0.05$). These data indicate that between 9 and 12 months of age, the 5xFAD mice ate less, had a lower body weight and were hypoactive compared to littermate WT controls. At 12 months of age the 5xFAD mice had less body fat and expressed less leptin, UCP-2, and hormone sensitive lipase mRNA than WT mice, indicating that fat loss due to abnormal levels of metabolic enzymes and hormones is the main contributor to weight loss in this mouse model. In addition, the 5xFAD mice expressed more UCP-1, indicating elevated thermogenesis and fat burn. Thus weight loss occurs largely because of WAT loss due to reduced adipogenesis (indicated by reduced hormone sensitive lipase mRNA expression) and elevated thermogenesis (due to elevated UCP-1 mRNA expression). These results may help to explain age-related weight loss in AD patients.

LIST OF ABBREVIATIONS USED

Aβ	Beta-amyloid
AD	Alzheimers disease
AgRP	Agouti related peptide
AICD	Amyloid precursor protein intracellular domain
Akt	Protein kinase B
APH-1	Anterior pharynx-defective phenotype-1
APP	Amyloid precursor protein
BACE-1	β-site amyloid precursor protein cleaving enzyme-1
BAT	Brown adipose tissue
B APP CTF	APP-β carboxyl terminal fragments
BBB	Blood brain barrier
Bcl-xL	B-cell lymphoma-extra large
BMI	Body mass index
BW	Body weight
CaMK2	Calcium/calmodulin-dependent protein kinase-2
CART	cocaine and amphetamine regulated transcript receptors
CK2	Casein kinase-2
CRH	Corticotrophin-releasing hormone
CSF	Cerebrospinal fluid
ELISA	Enzyme linked immunosorbent assay
ERK	Extracellular-signal-regulated kinases
FAD	Familial early-onset Alzheimer's disease
FI	Food intake
FIC	Food in cage
FOH	Food on hopper
FOXO4	Forkhead box O4
GLUT1, 3, 4	Glucose transporter type 1, 3, and 4
GSK-3β	Glycogen synthase kinase-3β
HIF-1α	Hypoxia inducible factor-1α

HSL	Hormone sensitive lipase
IL-6	Interleukin-6
IR	Insulin receptor
IRS	Insulin receptor substrate
JAK2	Janus kinase-2
JNK	c-Jun N-terminal kinases
LHA	Lateral hypothalamic area
LIPE	Lipase, hormone-sensitive
M	Mashed food
MAPK	Mitogen-activated protein kinase
MC4R	Melanocortin 4 receptor
MCH	Melanin concentrating hormone
MN-SOD	Manganese superoxide dismutase
mRNA	Messenger ribonucleic acid
α-MSH	Melanocyte-stimulating hormone
NCT	Nicastrin
NFT	Neurofibrillary tangles
NPY	Neuropeptide Y
ObRb	Leptin receptor
ORX	Orexins
OXY	Oxytocin
PDK	Phosphoinositide-dependent kinase
PEN-2	Presenilin enhancer-2
PIP2, 3	phosphatidylinositol 4, 5-bisphosphate-2
PKB	Protein kinase B
POMC	proopiomelanocortin
PPARγ	Peroxisome proliferator-activated receptor
PS	Presenilin
PVN	Paraventricular nucleus of the hypothalamus
qPCR	Quantitative polymerase chain reaction
Ras	Rat sarcoma

ROI	Region of interest
SD	Standard deviation
SOCS-3	Suppressor of cytokine signaling-3
SOS	Son of seven
STAT3	Signal transducer and activator of transcription-3
STZ	Streptozotocin
Tcf/Lef	Lymphoid enhancer factor
TNF-α	Tumor necrosis factor alpha
TRH	Thyrotropin-releasing hormone
UCP-1, 2	Uncoupling protein-1,2
WAT	White adipose tissue
WT	Wild-type

ACKNOWLEDGEMENTS

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CHAPTER 1 INTRODUCTION

1.1 ALZHEIMER'S DISEASE

1.1.1 Overview and economic impact of Alzheimer's disease

Alzheimer's disease (AD) is a neurodegenerative disease that appears in the latter half of life and has become an emerging pandemic that accounts for 70-90% of dementia cases (Luchsinger et al., 2009). It is predicted that by the year 2047 the prevalence of AD will quadruple, affecting 50% of those aged 85 and over (Luchsinger et al., 2009). Furthermore, 72% of patients with AD are women (Alzheimer's awareness month: spreading the word about the 72 percent, 2015). Longevity and higher rates of diabetes, obesity, and other risk factors associated with AD are thought to increase women's susceptibility to AD (Vina et al., 2010). Considering that the elderly are a growing proportion of the population, it is critical to develop a therapeutic intervention that may diminish the onset of this disorder. Costs for treatment are expected to increase to one trillion dollars per year, hence it is critical to develop effective interventions that may reduce the economic burden of this disease (Lee, 2011).

1.1.2 Types of AD: familial versus sporadic

There are two forms of AD which differ in prevalence, age of onset, and genetic risk factors. Familial early-onset AD (FAD) occurs in less than 1% of cases of AD and tends to emerge after 65 years of age (Deng et al., 2009; Zigmond et al., 2015). FAD has a large genetic component in that inherited autosomal dominant mutations are present in amyloid precursor protein (APP) leading to overproduction of full-length β-amyloid (Aβ) peptide from APP (Deng et al., 2009). Mutations have also been identified in presenilin-1 and 2 (PS), which lead to the overproduction of $A\beta_{42}$ (Correia et al., 2011). Consequently, these mutations affect β-secretase (i.e. BACE-1; β-site amyloid precursor protein cleaving enzyme-1) and γ-secretase (such as presenilin-1) proamyloidogenic cleavage of APP which increases Aβ proteins in the brain (Figure 1; De la Monte, 2012). These Aβ proteins, including $A\beta_{42}$, later oligomerize and become toxic (Figure 2; De la

Felice, 2009). Over 160 mutations in genes including APP and PS have been associated with the onset of FAD (Zigmond et al., 2015).

The second and most common form of AD is sporadic late-onset AD (SAD). Little is known about this form of AD except that type 2 diabetes, hyperinsulinemia, obesity, and insulin resistance, all of which suggest that metabolic syndrome (the inability to properly store and utilize energy) is a risk factor for SAD (Folch et al., 2013). Patients with sporadic AD tend to show symptoms after 65 years of age and tend to exhibit elevated levels of BACE-1 (an important β-secretase that cleaves the APP peptide; Zigmond et al., 2015). Overexpression and variants of Apolipoprotein E (APOE ε4) have also been identified as a risk fact for SAD (Chouraki and Seshadri, 2014). APOE is a component of multiple lipoproteins such as chylomicrons, high and very low density lipoproteins (Chouraki and Seshadri, 2014). In the periphery, it regulates transport of cholesterol and lipids throughout the body (Chouraki and Seshadri, 2014). In the central nervous system it is a ligand for low density lipid receptors, where APOE regulates catabolism, internalization, and binding of lipoproteins (Chouraki and Seshadri, 2014). Synapotogenesis, synaptic plasticity, and neuroinflammation are also regulated by this protein (Chouraki and Seshadri, 2014). Allelic variation of APOE ε4 can lead to increased plaque load, especially in SAD patients (Armstrong, 2013).

Chronic illness or infections may increase the chances for developing SAD as these lead to chronic inflammation in the brain, persistent activation of microglia, and dysregulation of pro-inflammatory cytokines (Lim et al., 2015). Mutations in the gene TREM2 are also a risk factor for SAD, as this gene mediates activation of microglia and macrophages, leading to dysregulation of the immune process, and reduced clearance of Aβ (Lim et al., 2015). Elevated activation of microglia, astrocytosis, and cerebral glucose hypometabolism are seen even before cognitive deficits emerge in AD patients (Nordberg, 2014).

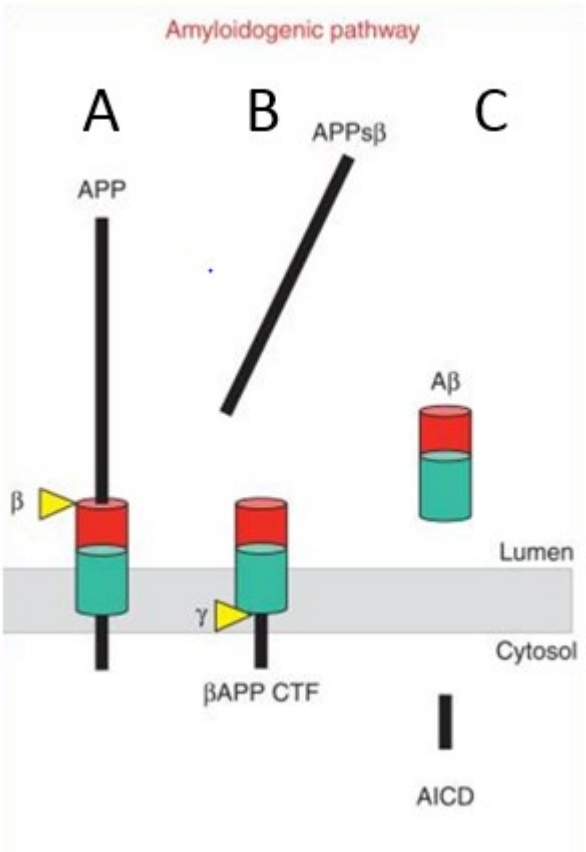

Figure 1 (A) Beta secretase (β secretase) cleaves amyloid precursor protein (APP) at several Aβ sites to (B) produce APP sβ and APP-β carboxyl terminal fragments (βAPP CTF (C). The βAPP CTF will then be cleaved by γ-secretases (Presenilin-1) to produce Aβ and APP intracellular domain (AICD). (From Haas et al., 2012).

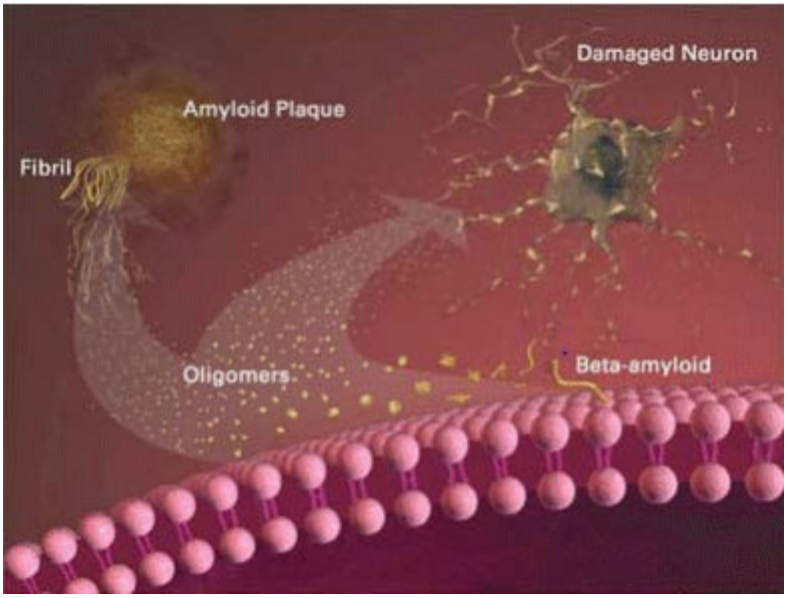

Figure 2 Cleaved beta-amyloid (Aβ) oligomerize, forming toxic oligomers that may directly damage neurons or further assemble into fibrils, and neurotoxic amyloid plaques. (From Goodman, 2015).

1.1.3 The pathology of AD

The neuro-pathological hallmarks of AD are extracellular Aβ plaques and intracellular neurofibrillary tangles (NFT) composed of hyper-phosphorylated tau proteins (Luchsinger et al., 2009). Both tangles and plaques are thought to contribute to disease severity because they are neurotoxic and damage neurons and synapses (Luchsinger et al., 2009). The tau hypothesis suggests that hyper-phosphorylated tau assembles in paired helical filaments that later aggregate into neurofibrillary tangles (Mohandas et al., 2009). These tangles may lead to cell death via microtubule disassembly and disrupted axonal transport (Mohandas et al., 2009; Figure 3).

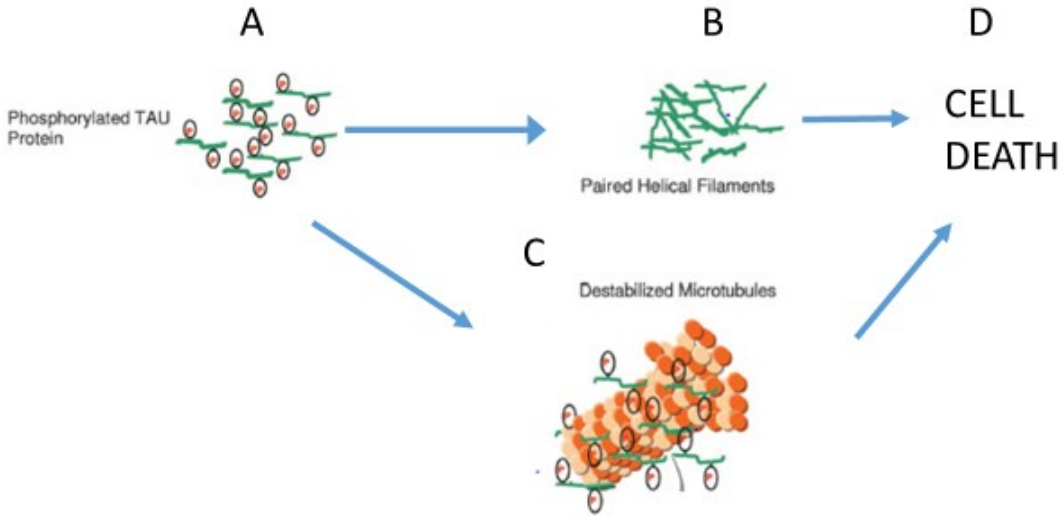

Figure 3 Depiction of the tau hypothesis. Hyper-phosphorylated tau (A), assembles into paired helical filaments (B) and destabilized microtubules (C), leading to cell death (D). (From Paula et al., 2009).

The amyloid cascade hypothesis states that the etiology of AD is primarily due to the abnormal cleavage of APP, leading to overexpression of $A\beta_{42}$ (Figure 4). Normally Aβ peptides are produced via proteolysis of APP proteins. The first cleavage is done by β-secretases (BACE-1), generally at the N-terminus (Lee, 2011). The second cleavage is made by intramembrane γ-secretase which is composed of four subunits: anterior pharnyx-defective phenotype-1 (APH-1), presenilin (PS), PS enhancer-2 (PEN-2), and nicastrin (NCT; required for secretase to recognize and bind to targets; Kim et al., 2012). These two enzymes facilitate the production of Aβ monomers. However, according to the amyloid cascade hypothesis, APP is abnormally cleaved by β and γ-secretases leading to the overproduction of $A\beta_{42}$ (Zigmond et al., 2015). This overproduction eventually leads to aggregation of $A\beta_{42,}$ resulting in cell death, dysregulation of calcium homeostasis, and disruption of mitochondrial function (Mohandas et al., 2009). Aβ plaques are toxic because they inhibit the phosphoinositide 3-kinase (PI3K) pathway, which mediates cell survival (Figure 5; De Felice et al., 2009). Consequently, the inhibition of the PI3K pathway leads to synapse loss and cell death (De Felice et al., 2009). Furthermore, these Aβ plaques attract microglia, which secrete inflammatory cytokines including tumor necrosis factor (TNF-α) and interleukin-6 (IL-6), which inhibit the PI3K pathway and induce cell death (Bomfim et al., 2012). Consequently, the amyloid cascade hypothesis overlaps with the inflammatory hypothesis in that Aβ plaques attract microglia and cause microglia to secrete the inflammatory cytokines such as TNF-α and IL-6 (Mohandas et al., 2009). However, the amyloid hypothesis has recently come under severe criticism and some researchers believe that it is not sufficient to explain the onset of Aβ (Castello and Soriano, 2014), or that it is a downstream result, rather than a cause, of AD (Drachman 2014).

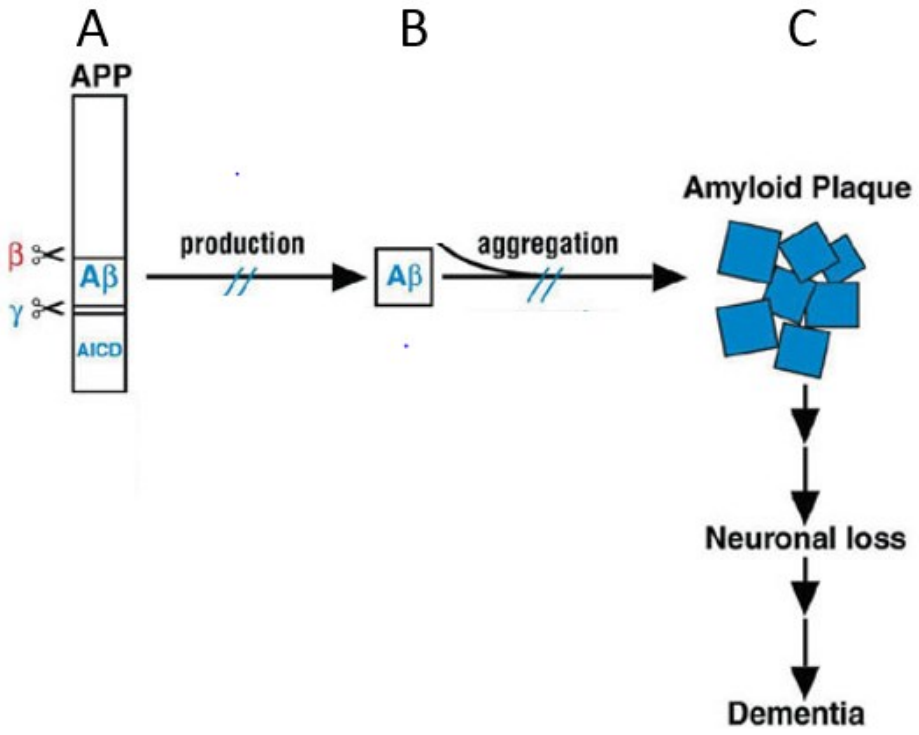

Figure 4 Depiction of the amyloid cascade hypothesis. (A) APP is abnormally cleaved by β and γ-secretases, which (B) leads to the overproduction of Aβ. Beta amyloid later aggregates and (C) forms plaques, leading to neuronal loss and dementia. (From Citron, 2002).

The progressive neuronal deterioration found in AD is not caused by Aβ monomers but by Aβ oligomers. In fact, the Aβ monomers are produced naturally in the cerebrospinal fluid (CSF) and brain vasculature and have neuroprotective functions, especially when trophic factors such as insulin or growth factors are absent (Giuffrida et al., 2009). Furthermore, these Aβ monomers activate insulin receptors (IR) and promote activation of the PI3K pathway, which promotes cell survival via several intracellular pathways (Giuffrida et al., 2009). One of these pathways is by inhibiting the enzyme GSK-3β (Glycogen synthase kinase), which in turn inhibits β-catenin (Giuffrida et al, 2009). If β-catenin is inhibited then it cannot bind to and activate transcription factors such as Tcf/Lef (Lymphoid enhancer factor). When Tcf/Lef is not activated cell differentiation, proliferation, and survival occurs (Giuffrida et al., 2009). Thus, Aβ in non-AD brains does not undergo extensive oligomerization as seen in AD (De Felice et al., 2009). Increased levels of Aβ oligomers may be due to impaired processing mechanisms (which are unknown) and protein misfolding (De la Monte, 2012). Elevated $A\beta_{42}$ may be due to alterations in the efficacy of BACE-1 cleavage of APP, leading to an elevated concentration of full-length Aβ peptide (Zigmond et al., 2015). $A\beta_{42}$ elevation may also be due to alterations in γ-secretase cleavage activity, leading to higher than normal concentrations of $A\beta_{42}$ (Zigmond et al., 2015). Misfolding of Aβ increases the probability of oligomerization that is seen in AD (Figure 2; De Felice et al., 2009). Consequently, this oligomerization of Aβ induces neurotoxic effects by antagonizing the effects of neural protective compounds such as insulin, and potentially leptin and ghrelin (all of which activate enzymes, such as Son of Sevenless (SOS) and Ras, that regulate the PI3K pathway illustrated in Figure 5), and thus increase the risk for neurodegeneration (De Felice et al., 2009). This neurodegeneration is achieved via activation of CaMKII (calcium/calmodulin-dependent protein kinase-2) and CK2 (Casein kinase-2), which down-regulates insulin receptors (IRs). The sites Aβ oligomers bind to in order to activate enzymes such as CaMKII and CK2, is not clearly understood (De Felice et al., 2009). Note that the PI3K pathway is likely not the only pathway affected in AD brains, as the MAPK pathway appears to also be affected. However, the majority of research has analyzed enzymes and proteins involved in the PI3K pathway of AD brains.

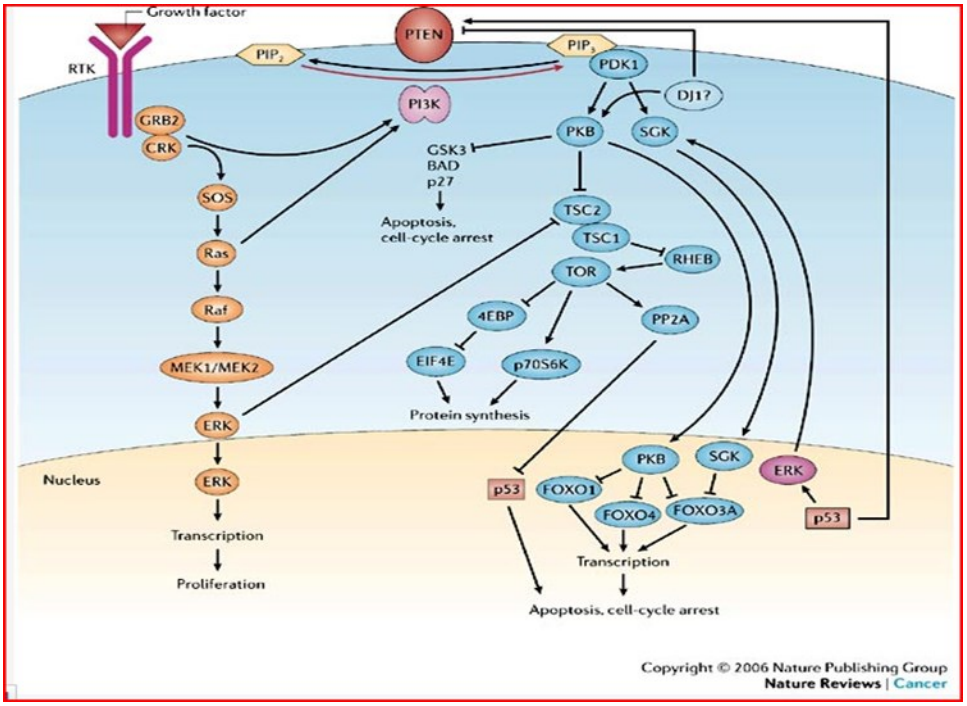

Figure 5 The PI3K pathway and its ability to prevent apoptosis. The prevention of apoptosis is best achieved by activating enzymes such as protein kinase B (PKB), which inhibits forkhead box O4 (FOXO4). PI3K is generally activated by Ras proteins (Rat sarcoma; a GTPase), which in turn is activated by Son of Sevenless (SOS; a guanine exchange factor). PI3K itself assists in the conversion of PIP2 to PIP3 (Phosphatidylinositol 4, 5-biphosphate-2, 3), which activates PDK (Phosphoinositide-dependent kinase) and PKB. This leads to inhibition of factors that stimulate apoptosis. Leptin, insulin and ghrelin innervate this pathway. (From Cully et al., 2006).

1.1.4 Central and peripheral behavioural symptoms in AD

Cognitive deficits are common symptoms of AD, and include learning, language and spatial memory impairments (Devi et al., 2012). As well as these cognitive deficits, AD results in peripheral symptoms such as motor deficits and weight loss (Adebakin et al., 2012; Devi et al., 2012; Fewlass et al., 2004). Mechanisms that contribute to weight-loss remain elusive (Gillete et al., 2007). A longitudinal study of 134 men and 165 women with AD found that 50% exhibited cognitive impairments accompanied by weight loss over the study (Barrett-Connor et al., 1996). Furthermore, 25% of the patients exhibited weight loss prior to the emergence of cognitive impairments, indicating that weight loss could be used to detect the onset of AD before cognitive symptoms were present (Barrett-Connor et al., 1996). Women with AD have a pronounced loss of fat mass and reduced body mass index (BMI), but show elevated lean body mass (Renvall et al., 1993). Although patients with AD may be hyperphagic, the presence of hyperphagia does not necessarily lead to weight gain (Keene et al., 1998). Weight loss reduces the quality of life for AD patients because it reduces their autonomy and life expectancy, increases the patient's risk of developing illnesses caused by opportunistic pathogens such as pneumonia, and weight loss is a predictor of mortality whereas weight gain in AD patients has a protective effect on the risk of mortality (Cronin-Stubbs et al., 1997, Hebert et al., 2010; White et al., 1998). Thus rapid and significant weight loss seen in AD patients is a sign of wasting and is highly associated with mortality (Guerin et al., 2009; White et al., 1998). Hence, additional research is warranted to determine the biological basis for body weight loss in AD.

1.1.5 Hormones and proteins involved in body weight and appetite and central metabolic disturbances in AD

The brains of AD patients' have metabolic disturbances which are similar to patients with obesity and type-2 diabetes, including disrupted brain insulin metabolism and impaired glucose metabolism (Correia et al., 2011; De la Monte et al., 2014; Lee, 2011). Furthermore, reduced concentrations of plasma leptin are found in AD patients (Lieb et al., 2009). These neuroendocrine impairments exacerbate the pathology and symptoms of AD and may lead to weight loss. Ishii et al. (2014) demonstrated that expression of orexigenic neuropeptide Y (NPY) and agouti related peptide (AgRP) was

abnormal in low leptin conditions in the Tg2576 mouse model of AD. This observation was abnormal as low leptin levels would normally induce the expression of these orexigenic neuropeptides. Furthermore, hyperpolarization of NPY and AgRP neurons in response to leptin were attenuated in this mouse model (Ishii et al., 2014). This indicates that leptin's efficacy in controlling signaling in NPY neurons is reduced and may explain the body weight loss seen in the Tg2576 mouse model (Ishii et al., 2014). Both insulin and leptin are hormones that regulate appetite, cell survival, and body weight and therefore abnormalities in the synthesis, storage, release, and or receptors of these hormones may underlie weight loss in AD (Craft et al., 2011).

1.1.5.1 Insulin: its effects and connection to AD

Insulin is a hormone made up of two trans chains, an A chain consisting of 21 amino acids and a B chain consisting of 30 amino acids, which is secreted by pancreatic β-cells in response to elevated plasma glucose levels, generally after a meal (Gardner and Shoback, 2011). Glucose diffuses across the β-cell plasma membrane via glucose transporters and once inside the pancreatic β-cells, glucose activates a series of intracellular signaling pathways that lead to calcium influx and inhibition of potassium channels, which leads to the synthesis and secretion of insulin (Gardner and Shoback, 2011). When it binds to the insulin receptor (IR), a tyrosine kinase receptor, insulin will promote the insertion of GLUT-4 receptors (glucose transporter type-4) into the plasma membrane (Figure 6; Gardner and Shoback, 2011). IRs are located in the liver, muscle, and adipose tissue (Gardner and Shoback, 2011), as well as in NPY expressing neurons in the hypothalamus, and the hippocampus (McNay et al., 2011). Generally, insulin will bind to the α-subunit of the IR and induce the β-subunit to autophosphorylate (Gardner and Shoback, 2011), which activates several phosphatases and kinases such as IRS (insulin receptor substrate) and PI3 kinase (Correia et al., 2011). The PI3 kinase enzymes convert PIP2 to PIP3, which in turn will activate protein kinase B (Akt), a kinase within the PI3K pathway (Figure 6; Gardner and Shoback, 2011). Once activated, the PI3K pathway leads to the insertion of GLUT-4 channels in the plasma membrane, and the inhibition of glycogen synthase kinase-3β (GSK3β), which then allows glycogen synthase to convert glucose to glycogen (Gardner and Shoback, 2011). Whether the tissue is muscle, fat, or liver, insulin induces the same effect; it promotes the conversion of

glucose to glycogen and increases the net intake of glucose within these particular tissues (Gardner and Shoback, 2011).

At the hypothalamus, insulin has anorexigenic effects as it inhibits appetite and promotes energy expenditure by inhibiting NPY and AgRP neurons from responding to ghrelin (Figure 7; Maejima et al., 2011). NPY/AgRP neurons are located in the arcuate nucleus of the hypothalamus and inhibit the activation of melanocortin 4 receptors (MC4R), located in the paraventricular nucleus of the hypothalamus, leading to increased food intake and decreased energy expenditure (Barsh and Shwartz, 2002). However, insulin may also directly innervate neurons expressing proopiomelanocortin / cocaine and amphetamine regulated transcript peptide (POMC/CART) in the arcuate nucleus, which in turn activate MC4Rs (Barsh and Schwartz, 2002). This leads to an increase in energy expenditure. Also NPY/AgRP and POMC/CART neurons regulate neurons in the lateral hypothalamic area (LHA; Figure 8; Ahmia and Osei, 2004). These neurons contain melanin concentrating hormone (MCH), and orexins (ORX), both of which are orexigenic and activate cerebral cortex feeding centers (inducing feeding; Ahima and Osei, 2004). Both POMC/CART and NPY/AgRP inhibit these neurons.

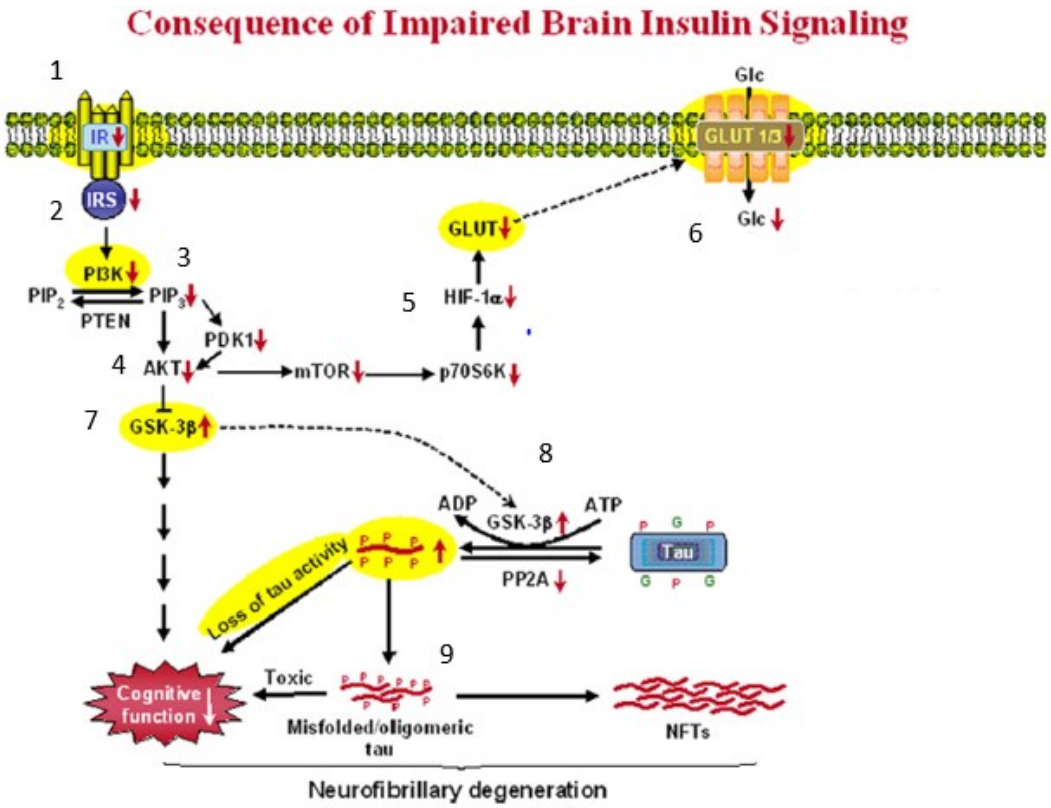

Figure 6 Insulin is important for regulating GLUT-1, 3, 4 upregulation and tau phosphorylation. Insulin binds to insulin receptors (1), which activates IRS and PI3K kinases (2 and 3). This leads to the activation of Akt (4), which activates hypoxia inducible factor-1α (HIF-1α) and inhibits GSK-3 β (7). Activation of HIF-1α leads to the upregulation of GLUT-1, 3, 4 on the cell membrane (6). If insulin signaling in the brain is impaired, then GSK-3 β will be activated, due to lack of inhibition by Akt, and will directly phosphorylate tau (8). This leads to the hyperphosphorylation of tau (9). Hyperphosphorylated tau will oligomerize and form toxic neurofibrillary tangles. (From Deng et al., 2009).

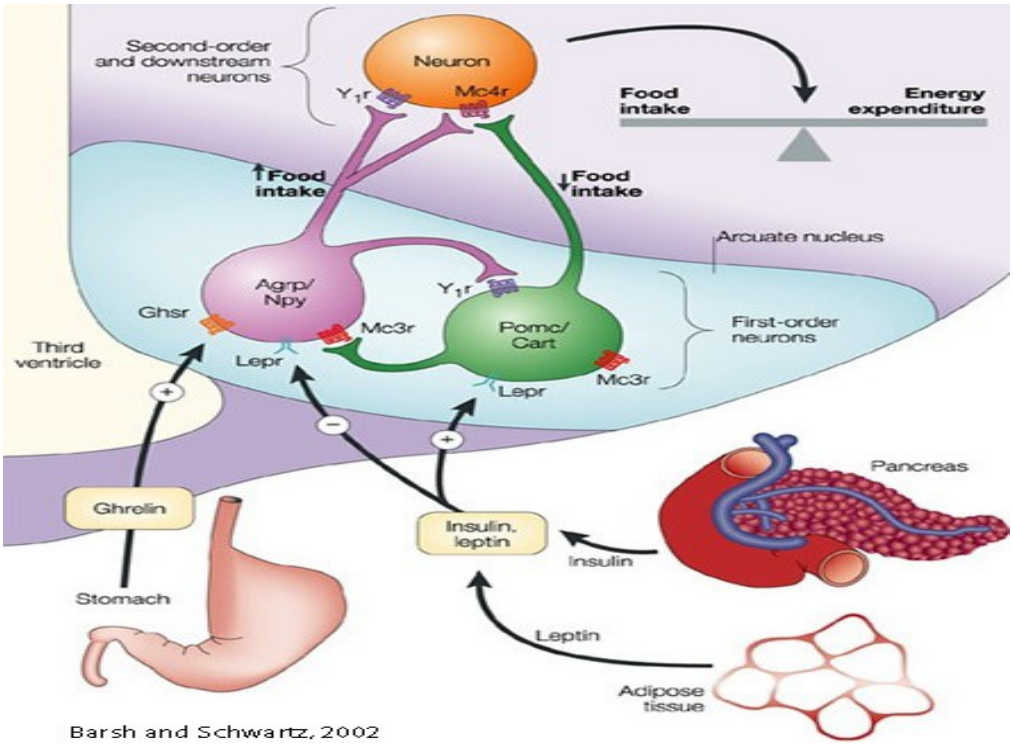

Figure 7 Actions of insulin, leptin, and ghrelin in the hypothalamus. Insulin and leptin excite POMC/CART neurons, which activate MC4R receptors and inhibit AgRP/NPY neurons. The net effect is increase in energy expenditure and a decrease in food intake. Insulin is secreted by β-cells in the pancreas, and leptin is secreted by adipocytes. Ghrelin, secreted by the stomach, activates AgRP/ NPY neurons and this increases food intake and decreases energy expenditure. AgRP/NPY neurons antagonize MC4R. (From Barsh and Schwartz, 2002).

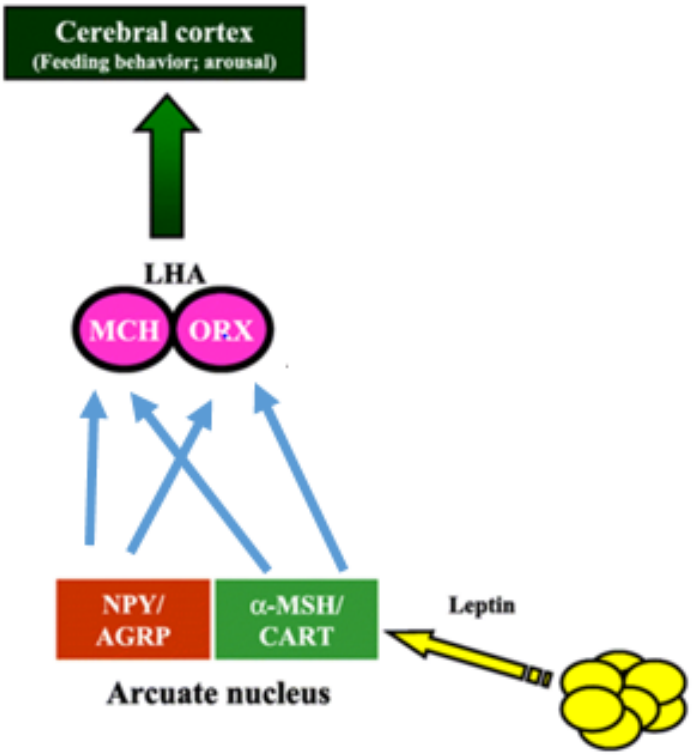

Figure 8 Melanocyte-stimulating hormone (CART/α-MSH) or POMC neurons in the arcuate nucleus of the hypothalamus will inhibit orexin (ORX) and melanin-concentrating hormone (MCH), found within the lateral hypothalamic area (LHA). NPY and AgRP inhibit ORX and MCH neurons. ORX and MCH initiate feeding behaviour via neurons in the cerebral cortex. (From Ahima and Osei, 2004).

In the brain, insulin promotes cell survival, affects learning and memory, and supports neural plasticity via activation of IRs located in several regions in the brain, most importantly in the hippocampus (Craft et al., 2011). Activation of IRs leads to activation of the PI3K pathway, which increases levels of Akt (Talbot et al., 2012). This pathway promotes cell survival by various means. One of these includes inhibition of FOXO1, a transcription factor that promotes apoptosis, via inhibition of PKB (Cully et al., 2006). Akt is also critical for the preservation of neurons by inhibiting GSK-3 (Talbot et al., 2012). GSK-3 hyperphosphorylates tau proteins, leading to their impaired function and misfolding (De la Monte, 2012). Also GSK-3, inappropriately activates β-secretases, increasing the concentration of Aβ (Figure 9; Farooqui et al., 2012).

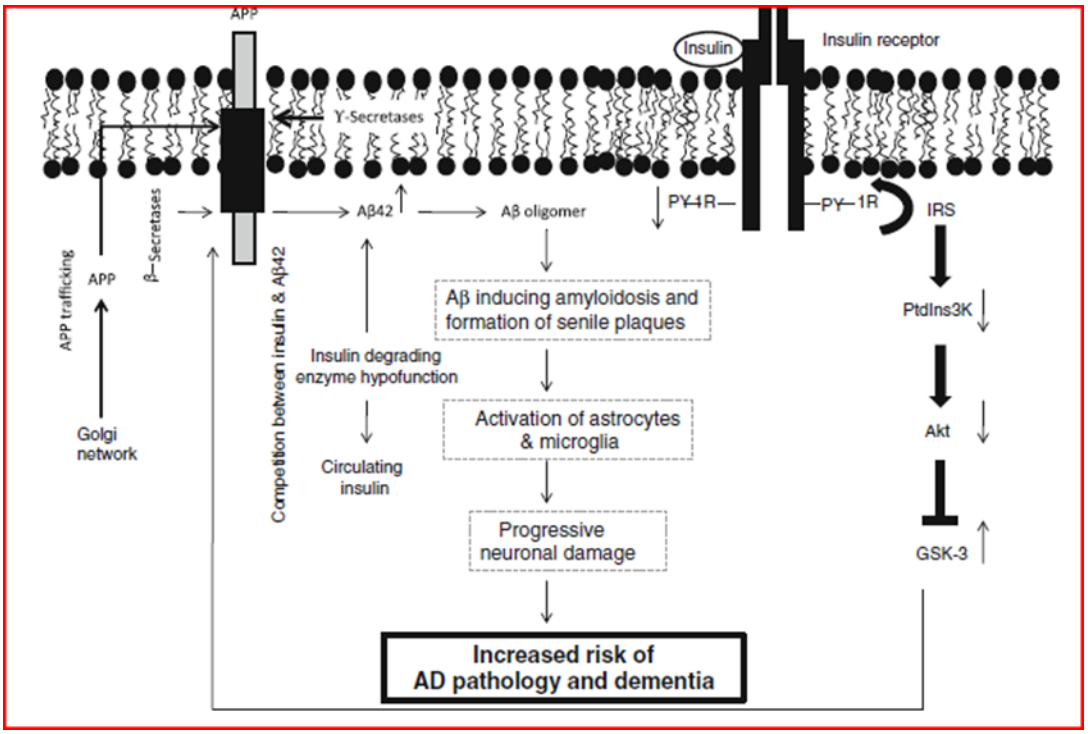

Figure 9 Insulin is an important neurotrophic factor that mediates cell survival by activating the PI3K pathways. Upon activation of the insulin receptor, insulin receptor substrate (IRS) and PI3K are activated. Consequently, Akt is phosphorylated and inhibits GSK-3. This leads to reduced activation on β-secretases, leading to reduced $A\beta_{42}$ production. During insulin resistance, GSK-3 is activated due to a lack of inhibition from Akt (as PI3K and IRS are not activated). GSK-3 results in increased activity of β-secretase, which will lead to an increase in the level of $A\beta_{42}$, as β and γ-secretases cleaves membrane bound APP produced from the Golgi network. $A\beta_{42}$ will oligomerize, inducing neuroinflammation and cell death. Lack of insulin degrading enzyme activation leads to increased Aβ oligomerization and levels of circulating insulin (From Farooqui, 2012).

Another pathway activated by insulin receptors in the brain is the mitogen-activated protein kinase (MAPK) pathway, which facilitates long term potentiation and cell growth via extracellular-signal-regulated kinase (ERK; Correia et al., 2011). ERK generally activates several transcription factors that promote cell growth and survival (Correia et al., 2011). Consequently, both MAPK and PI3K pathways are critical in the brain for neuronal survival and growth. Insulin is a trophic factor that activates these pathways, particularly PI3K (Correia et al., 2011).

In AD, there appear to be abnormal response to insulin in both neuronal and peripheral tissues (Luchsinger et al., 2009). Within the brain of AD patients and AD mouse models, there is significant resistance to insulin (Luchsinger et al., 2009). There are several theories as to why this may occur. One of these involves the inhibition of IR by Aβ oligomers (De Felice, 2009). These oligomers activate unknown receptors, which in turn activate CamKII and CK2, which induce internalization of IRs (De Felice, 2009). The second theory is that hyperinsulinemia found in the periphery may lead to downregulation of IRs in the CNS and reduce brain derived secretion of insulin (De la Monte, 2012). In addition, insulin transporters reduce the rate of transport of insulin across the blood brain barrier when plasma levels of insulin are high (the majority of insulin within the brain originates from the periphery; Banks, 2004). Patients with AD tend to have elevated levels of plasma insulin and reduced levels of insulin within the brain (Craft et al., 1998). Insulin allows glucose to enter cells via up-regulating GLUT-1, 3, 4 receptors (Figure 4). Consequently, glucose metabolism in cells is hindered if insulin resistance occurs. Therefore, the glucose transporters GLUT-1 and GLUT-3, which ensure that glucose enters neurons to promote neuronal survival, are reduced in the brains of AD patients, leading to reduced transport of glucose into neurons and increased cell death (Lui et al., 2008). It has been shown that intranasal injections of insulin, rosiglitazone (an insulin sensitizing agent), and metformin (another insulin sensitizing agent) all reduce cognitive deficits in AD mice (De la Monte, 2012). The reduction of insulin resistance via these pharmaceuticals alleviates AD symptoms, suggesting insulin may act as a neurotrophic factor to facilitate neuronal survival and may inhibit the onset of AD.

1.1.5.2 Leptin and hormone sensitive Lipase: their connection to Alzheimer's Disease

Leptin is a 16 KDa protein produced by adipocytes and leptin may cross the blood brain barrier (BBB) via a leptin transporter (Coppari and Bjørbæk, 2012). Leptin is classified as a cytokine, but also functions to regulate energy metabolism and appetite (Barsh and Schwartz, 2002). Leptin binds to the leptin receptor type b (ObRbs) located in the arcuate nucleus of the hypothalamus and the hippocampus, where it has similar effects to insulin: a decrease in food intake and increase in energy expenditure via activation of the neurons within the PVN and inhibition of neurons in the LHA (Ahima and Osei, 2004). The consequence of this activation of the PVN, is stimulation of glucose uptake in the liver and muscle, and a decreased insulin production and secretion in the pancreas via nerves of the parasympathetic nervous system (Ahima and Osei, 2004).

In the brain, leptin binds to ObRb receptors located primarily in the hippocampus (Doherty, 2011). These leptin receptors are similar to IR in that they are tyrosine kinases (a receptor that autophosphorylates itself to activate secondary messengers) which activate the PI3K pathway (Doherty, 2011). Once activated, leptin receptors autophosphorylate their intracellular domain, which activates proteins such as Janus kinase-2 (JAK2) and signal transducer and activator of transcription 3 (STAT3; Figure 10; Garza et al., 2008). The protein STAT3 dimerizes and activates the transcription of several proteins such as B-cell lymphoma-extra large (Bcl-xL) and manganese superoxide dismutase (MN-SOD), both of which stabilize the mitochondria (Guo et al., 2007). More importantly, Bcl-xL prevents the activation of caspase enzymes, hence preventing apoptosis. JAK2 also activates the PI3K pathway and has similar neuroprotective effects as insulin (Guo et al., 2007). As well as being neuroprotective, leptin activates pathways underlying learning and memory processes (Guo et al., 2007).

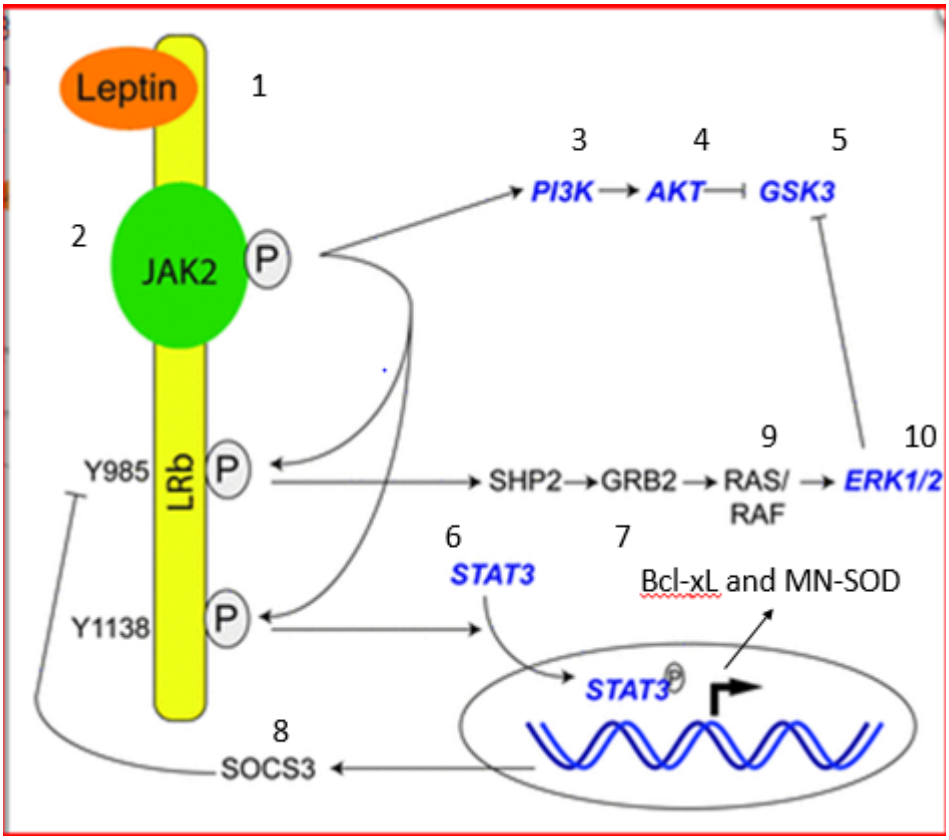

Figure 10 Leptin receptors are tyrosine kinases that autophosphorylate and activate enzymes such as Janus kinase-2 (JAK2). When leptin binds to the leptin receptor (1), JAK2 is activated via autophosphorylation (2). JAK2 in turn activates PI3K and Akt (3 and 4). Akt inhibits GSK-3 (5). JAK2 also activates signal transducer and activator of transcription-3 (STAT3; 6), which induces the transcription of enzymes such as MN-SOD and Bcl-xL, both of which promote mitochondrial stability and prevent apoptosis (7). Suppressor of cytokine signaling-3 (SOCS3) is also produced and acts as a negative feedback signal upon the leptin receptor, inhibiting autophosphorylation (8). JAK2 will also stimulate Ras (9), a GTPase that activates a kinase, and ERK 1/2 which inhibits GSK-3 (10). (From Lee, 2011).

About 50% of AD patients have reduced plasma levels of leptin, and the reduction of this neurotrophic factor in the brain may increase the likelihood of neurodegeneration (Lieb et al., 2009). Furthermore, low levels of leptin may be a risk factor for AD (Lieb et al., 2009).

Hormone sensitive lipase (HSL) is a marker for adipose catabolism and is found in white and brown adipose tissue and regulates free fatty acid secretion into the circulation (Haemmerle et al., 2001). Consequently, activation of HSL leads to leaner body mass (Cummings et al., 1996). Although the connection of HSL to AD is unclear, mutations on the lipase, hormone-sensitive (LIPE) gene encoding HSL lead to a defect in the catabolism of triglycerides to fatty acids and glycerol (Zechner, 2014). Furthermore, circulating fatty acids deposit in the liver rather than in adipocytes (Zechner, 2014). This leads to fat tissue loss (Zechner, 2014). Increased expression for HSL may indicate reduced adipogenesis.

1.1.5.3 UCP-1 and UCP-2: Their effects and connection to AD

One hypothesis as to why weight loss occurs in AD is hypermetabolism. Although hypermetabolism has yet to be identified in AD patients, some mouse models of AD, such as 3x-TgAD, mice exhibit hypermetabolism (Poehlman and Dvorak, 2000; Knight et al., 2010). The 3x-TgAD mice consumed more oxygen (24%) and produced more carbon dioxide (29%) than their wild-type (WT) counterparts, supporting the hypothesis that the 3x-TgAD mice are in an elevated metabolic state (Knight et al., 2010). One marker for hypermetabolism is uncoupling protein-1 (UCP-1) on the inner mitochondrial membrane in brown adipose tissue (BAT; Yo et al., 2013). Elevated expression of UCP-1 has been associated with thermogenesis (converting adenosine triphosphate to heat in order to prevent hypothermia) and fat burn (Yo et al., 2013). Furthermore, humans and mice that have elevated metabolism exhibit elevated activation of BAT and elevated UCP-1 expression (Yo et al., 2013). However, AD patients show no elevated expression of UCP-1 (Cornelius et al., 2013). Nonetheless it is important to know if UCP-1 is elevated in mouse models of AD that are hypermetabolic.

UCP-2, like UCP-1, is a mitochondrial protein found in the inner mitochondrial membrane of white adipose tissue (Vidal-Puig et al., 1997). Its function is similar to UCP-1 in that it separates oxidative phosphorylation from adenosine triphosphate

synthesis. This results in the production of heat. Consequently, UCP-2 also regulates thermogenesis (Arsenijevic et al, 2000). Furthermore, UCP-2 reduces the production of reactive oxidative species (Arsenijevic et al, 2000). Indeed, knockout mice lacking UCP-2 display elevated concentrations of reactive oxidative species (Arsenijevic et al, 2000). UCP-2 is up-regulated by leptin (Zhang et al., 2008). This relationship indicates that if little leptin is present, then expression of UCP-2 should be reduced.

1.1.6. AD as a metabolic disorder

As the studies above suggest, AD patients have metabolic abnormalities. Indeed, patients with AD exhibit lowered plasma levels of leptin, elevated plasma levels of insulin, and reduced cerebral glucose metabolism (Lieb et al., 2009; Nordberg, 2014; Zeltser et al., 2012). Leptin and insulin are neurotrophic factors that promote cell survival and prevent cell death and also regulate food intake and body weight (Doherty, 2011; Zeltser et al., 2012). Due to a brain resistant insulin and leptin state these hormones cannot promote cell survival effectively, increasing the chances of cell death (Lieb et al., 2009; Zeltser et al., 2012). Furthermore, abnormalities in these hormones may alter food intake and body weight. Glucose is the main neuronal energy source, reduction in glucose transport leads to cell death and may exacerbate neural degeneration seen in AD (Lui et al., 2008).

UCP-1 and UCP-2 regulate thermogenesis whereas HSL regulates fat catabolism (Yo et al., 2013; Vidal-Puig et al., 1997; Zechner et al., 2014). Abnormalities in these proteins may explain body weight loss. Consequently, this study wishes to investigate whether the abnormalities listed above are seen the 5xFAD mouse model which exhibits progressive weight loss.

1.2 Mouse models of AD

There are multiple mouse models of AD that have been genetically engineered to develop symptoms of AD. These mouse models include Tg2576, 3xTg-AD, 5xFAD and many more. Mouse models differ in level of $A\beta_{42}$ concentration intra and extra neuronally, and the presence of tau tangles (Bilkei-Gorzo, 2013). These mouse models differ in face validity (same symptoms seen in model and disease), construct validity (same pathological mechanisms seen in model and disease), and predictive validity (same

effectiveness of treatment between model and disease; Bilkei-Gorzo, 2013). All mouse models of AD develop age-related cognitive impairments (Bales, 2012) and the focus has been to use these mouse models to study the cognitive deficits and histological hallmarks of AD (Webster et al., 2014). Very few studies have examined the problem of age-related weight loss in AD.

1.2.1 The purpose and limitations of AD mouse models

Transgenic mouse models such as the 3xTg-AD, Tg2576 and 5xFAD mice have been valuable in studying the molecular and behavioural processes that occur in AD (Bales, 2012). Furthermore, these mouse models are useful to assess the efficacy of novel treatments for AD, such as sominone drug (a steroid that improves cognition and synaptic reconstruction in 5xFAD mice) and insulin intranasal injection studies (Joyashiki et al., 2010). Limitations to the use of these mouse models are that some do not develop NFTs as seen in AD brains and some do not show overt neurodegeneration (Bales, 2012). Furthermore, mouse models that tend to show overt neurodegeneration, have overexpressed APP mutations that lead to toxic intraneuronal $A\beta_{42}$ (Bales, 2012). Patients with AD do not develop extensive intraneuronal accumulation of AB_{42} (Bales, 2012). Many mouse models, such as the 5xFAD, develop from mutant transgenes (i.e. APP and PSEN) injected into embryos resulting in elevated expression of $A\beta_{42}$. Consequently, transgenic mice begin to produce Aβ in the womb. For some mouse models it is only at 1.5 months of age that these $A\beta_{42}$ become detectable. This is when the mice are pubescent but patients with AD do not develop this pathology during puberty (Arnoud, 2010; Lifespan as a biomarker, 2011). Hence age of pathology onset differs between mouse models and AD patients. Finally, comparing effects from treatments given to AD mice to human AD patients is challenging as many of these treatments, while successful in mice, are unsuccessful in humans (Bales, 2012; Webster et al., 2014).

1.2.3 Rationale for using the 5xFAD mouse model and issues with current studies using 5xFAD mouse model of AD

We chose female 5xFAD mice because they have been found to have high face validity of AD (Bilkei-Gorzo, 2013; O'Leary, 2013). Not only do these mice exhibit AD-like symptoms earlier than most mouse AD models but they are one of the few that

develop progressive weight-loss (Jawhar et al., 2010). Because of this phenotype, we were interested in investigating the mechanisms that contribute to this weight-loss. Females were chosen as subjects of this study as most AD patients are women. Furthermore, the usefulness of the 5xFAD mouse for studying metabolic abnormalities in AD has been demonstrated by researchers who injected Streptozotocin (STZ) in 5xFAD mice to mimic diabetes-like symptoms (Devi et al., 2012). STZ is a toxin that specifically targets β-cells in the pancreas, resulting in a dramatic drop in both plasma and brain insulin levels (Devi et al., 2012). 5xFAD mice given STZ showed increased cognitive decline compared to untreated 5xFAD mice, thus creating an AD mouse co-morbid with diabetes (Devi et al., 2012). These results indicate that metabolic disorders like type-1 and 2 diabetes may exacerbate AD symptoms in the 5xFAD mouse (Devi et al., 2012).

Studies using the 5xFAD mouse model focus mostly on the cognitive, behavioural, and histological impairments rather than the peripheral and metabolic impairments. Studies about weight loss in this AD mouse model have not attempted to investigate factors that influence weight-loss (Jahwar et al., 2010; O'Leary, 2013). The purpose of the present thesis is to investigate the behavioural and endocrine mechanisms that may lead cause this weight loss.

1.2.4 Genome and pathology of the 5xFAD mouse model of AD

The 5xFAD mice have mutant human APP (695) with three mutations, Sweden (K670N, M671L), London (V717I), Florida (I716V), and two human PS1 mutations (M146L and L286V; Oakley et al., 2006). The expression of these transgenes is controlled by neural-specific elements of the mouse Thy1 promoter. The net effect of these mutations is an increase in concentration of Aβ monomers that later oligomerize and induce toxic effects (Bomfim et al., 2012). The accumulation of Aβ occurs both intraneuronally and extraneuronally (Bales, 2012). The 5xFAD mouse is a reliable and valid mouse model of AD as AD symptoms develop more rapidly relative to other mouse models (Jawhar et al., 2010). The first of these Aβ deposits occur at 2 months of age and by 6 months of age extensive Aβ deposits are observed. Consequently, these plaques may be seen in the hippocampus and motor cortex, regions that influence memory and motor control (O'Leary, 2013). Neuroinflammation is commonly found in the 5xFAD mouse model (Bilkei-Gorzo, 2014). This involves elevated levels of TNF-α and IL-6,

exacerbating cell death (Bilkei-Gorzo, 2014). Hyperphosphorylated tau and NFT are absent in this mouse model.

1.2.5 Behavioural abnormalities in 5xFAD mice

By 4 months of age, 5xFAD mice show cognitive deficits in the object recognition test (Joyashiki et al., 2011). By 9 months of age the 5xFAD mice exhibit motor impairments on the rotorod (O'Leary, 2013). Between 12-13 months of age, the 5xFAD mice show deficits in grip strength and balance (O'Leary, 2013). These motor impairments may stem from plaque load in regions of the brain that control motor behaviour (O'Leary, 2013). Acquisition and reversal learning, using the Morris water maze, is also impaired in these mice between 6-9 months of age (O'Leary, 2013). Cognitive impairments are likely due to plaque load in regions that regulate memory processing, such as the hippocampus (O'Leary, 2013). Age-related weight loss in this model begins to show at 9 months of age (Jawhar et al., 2010). Weight loss is also seen in AD patients and because of the publicity of research the factors underlying age-related weight loss, it was decided to investigate these in this thesis.

1.2.6 Purpose of this study

1.2.6.1 Objectives

The purpose of this study was to measure the behavioural and metabolic factors that affect food consumption and body weight from 3-12 months of age in the 5xFAD mouse model of AD, and investigate the possible behavioural and endocrine mechanisms underlying this weight loss. In this thesis, six questions are investigated:

1) Does food presentation affect weight loss?
2) Is there an issue with food intake?
3) Do the frailty scores of the mice change under different feeding conditions?
4) Does hyperactivity contribute to weight loss?
5) Is there tissue atrophy during weight loss?
6) Are the metabolic factors that regulate body and appetite dysregulated?

1.2.6.2 Hypotheses tested and outline of experiments

1) We exposed cross-sectional mice 9, and 12 months of age and longitudinal mice at 3, 6, 9, and 12 months of age for 7 days to three different food presentations: food on hopper, food in cage, and mashed food. During this time we measured body weight. This was an attempt to answer whether food presentation affected body weight. Given that 5xFAD mice are good representations of the clinical symptoms seen in AD patients, we predicted that feeding behaviour will increase and body weight will be reduced in 5xFAD mice.
2) Cross-sectional mice 9, and 12 months of age and longitudinal mice at 3, 6, 9, and 12 months of age were exposed for 7 days to three different food presentations: food on hopper, food in cage, and mashed food. During this time we measured food intake. This was an attempt to answer whether food presentation affected amount of food consumed. We predicted that the method of food presentation will affect amount eaten in that more accessible food will lead to increased food consumption in 5xFAD mice.
3) Frailty scores were measured at 12 months of age for cross-sectional and longitudinal mice. They were exposed to three different food presentations for 7 days: food on hopper, food in cage, and mashed food. At the end of the 7 days frailty was measured using the mouse frailty assessment form to see if frailty reduced as food became more accessible. We believed different feeding conditions will alter frailty in that frailty scores would be reduced after introducing different food presentations: food on hopper, food in cage, and mashed food
4) All mice were video recorded at 12 months of age and analyzed for frequency of grooming, rearing, jumping, climbing, and freezing. The sum of these frequencies provided insight into the activity levels of the 5xFAD mice. We believed 5xFAD would be hypoactive due to their deficits in motor skills.
5) Muscle, white adipose tissue, and brown adipose tissue mass were recorded at 12 months of age in all mice. Results provided information on whether 5xFAD mice developed age-related sarcopenia, fat loss, or both. We predicted fat would be reduced and lean muscle mass would remain unchanged.

6) Blood samples were taken and analyzed for plasma insulin and glucose levels. White and brown adipose tissue were collected and analyzed for several proteins indicative of adiposity, adipogenesis, and thermorgenesis. These results were expected to highlight any dysregulation of hormones and proteins that regulate body weight and appetite. We predicted that there would be dysregulation of insulin and that we would observe leptin, hormone sensitive lipase, and uncoupling protein-2 mRNA levels to drop, uncoupling protein-1 mRNA levels to rise, and insulin levels to rise.

Chapter 2 will focus on questions 1-3 and chapter 3 will focus on question 4-5. This research is important with respect to clinical issues seen in AD patients.

CHAPTER 2 AGE-RELATED CHANGES IN FEEDING, BODY WEIGHT, ACTIVITY, AND FRAILTY IN 5XFAD MICE

2.1 ABSTRACT

The 5xFAD mouse is a double transgenic model of Alzheimer's disease (AD), which carries an amyloid precursor protein (APP) transgene with three mutations, and a presenilin-1 transgene with two mutations. These mutant transgenes act additively to produce Aβ plaques as early as two months of age. As occurs in human AD patients, age-related weight loss is seen in 5xFAD mice. We investigated age-related changes in feeding behaviour and body weight in female 5xFAD mice and their WT (C57BL/6JxSJL/J F1) littermates from 3 to 12 months of age. Activity levels and frailty were measured at 12 months of age. At 9 and 12 months of age WT mice ate more and weighed more than 5xFAD mice ($p<0.05$). Levels of grooming and jumping did not differ, but 5xFAD mice showed less climbing and rearing than WT mice ($p<0.05$), and spent more time remaining still than WT mice ($p<0.05$). The 5xFAD mice had higher frailty scores than WT mice ($p<0.05$). Different food presentations, food on hopper, food in cage, or mashed food had no effect on body weight or on frailty score ($p>0.05$). Our data indicate that age-related weight loss in 5xFAD mice may be partially explained by reduced food intake. We also conclude that the 5xFAD mice are hypoactive compared to WT mice when behaviours are complex and require a significant amount of energy, and that frailty is elevated in the 5xFAD mice and is not influenced by food presentation. These results suggest that the 5xFAD mice may have a metabolic disorder which leads to age-related weight loss.

2.2 Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder that occurs in individuals after 60 years of age (Minati et al., 2009). AD accounts for 70-90% of human dementia cases and it is predicted that by the year 2047, the prevalence of AD will quadruple (Luchsinger et al., 2009). Considering that the elderly are a growing proportion of the population, it is critical to develop methods to detect AD before symptoms emerge and to develop novel therapeutic interventions to reduce the severity of this disorder. As well as cognitive deficits, AD patients show peripheral symptoms such as weight loss and motor rigidity (Buchman and Bennett, 2011; Devi et al., 2012; Fewlass et al., 2004). Mechanisms that contribute to weight-loss remain elusive (Gillette et al., 2007). A longitudinal study of 134 men and 165 women with AD found that 50% of patients exhibited cognitive impairments accompanied by weight loss over the study (Barrett-Connor et al., 1996). Furthermore, 25% of the patients exhibited weight loss prior to the emergence of cognitive impairments, indicating that weight loss could be used to detect the onset of AD before cognitive symptoms are present (Barrett-Connor et al., 1996). Feeding abnormalities can occur in AD, with some patients displaying either hyperphagia or hypophagia. However, hyperphagic AD patients do not necessarily gain weight (Keene et al., 1998). AD patients that exhibit hypophagia, experience a loss of appetite and lose weight (Sergi et al., 2013). Weight loss reduces the quality of life for AD patients because it reduces their autonomy and life expectancy (Adebakin et al., 2012; Hebert et al., 2010). Hence, additional research is warranted to determine the biological basis for alterations in feeding behaviour and body weight-loss in AD.

Transgenic mouse models have been valuable in studying the etiology of molecular and behavioural processes that occur in AD (Shineman et al., 2001; Webster et al., 2014). These mouse models are useful to assess the efficacy of proposed novel treatments for AD. Most of these mouse models have been used to study cognitive deficits rather than weight-loss and other symptoms which are seen in AD. The double transgenic 5xFAD mouse model expresses age-related weight loss similar to that seen in patients with AD (Jawhar et al., 2010). These mice have mutant human APP with three mutations (Sweden, London, Florida) and two PS1 mutations (M146L and L286V; Oakley et al., 2006). The net effect of these mutations is an increase in the concentration

of Aβ monomers that later oligomerize and induce toxic effects (Bomfim et al., 2012). The 5xFAD mouse is a reliable and valid mouse model of AD as AD symptoms develop more rapidly relative to other mouse models (Jawhar et al., 2010). The 5xFAD mice have been used for studying disease mechanisms underlying cognitive deficits, but no research to date has used 5xFAD mice to assess food intake and weight loss symptoms commonly seen in AD patients.

Frailty is defined as increased susceptibility to problematic health issues for individuals of identical chronological ages (Feridooni et al., 2015). Tests for frailty include non-invasive test batteries that assess the number and severity of deficit accumulations for a variety of behaviours such as vision, grip strength, inflammation etc. (Feridooni et al., 2015). An increase in frailty scores has been correlated with ageing and mortality (Rockwood et al., 2015). Seniors who score 0.6 and higher on the frailty index have an 80% chance or greater of dying in the next few months (Rockwood et al., 2015). Aged mice also exhibit an age-related increase in frailty scores, and the relationship between frailty and ageing is similar in mice and humans (Whitehead et al., 2014). To date no project has attempted to investigate frailty in the 5xFAD mouse model or to determine whether age-related changes in body weight influence frailty scores.

This set of experiments aims to examine the relationship between feeding behaviour, activity levels, body weight, and frailty in the 5xFAD mice. We will examine the relationship between food intake, food presentation, hyperactivity, and weight loss seen in this mouse model. We will also measure frailty in this mouse model and to see if feeding and body weight influence frailty scores.

2.3 METHODS

2.3.1 Animals

A total of 44 5xFAD and 35 WT female mice were used in this study, 29 5xFAD mice and 20 WT mice were randomly selected and used in the cross-sectional study and 15 5xFAD mice and 15 WT mice were randomly selected and used for the longitudinal study. Male 5xFAD mice were purchased from Jackson labs (Bar Harbour, Maine; stock number 034840-Jax; model number: B6SJLT-Tg (APPSwFlLon,

PSEN1*M146L*L286V) 6799Vas/Mmjax), and bred in our lab with female wild-type C57BL/6JxSJL/J F1 mice and the F2 offspring were used in the present study. We used female 5xFAD mice because majority of AD patients are women and because the majority of male 5xFAD mice die before 15 months of age. For the 15 WT and 15 5xFAD mice tested longitudinally, we measured food intake and body weight at 3, 6, 9, and 12 months of age. The majority of tests were done at 12 months of age because significant weight discrepancy between WT and 5xFAD occurs at this age. Between weaning at 22 days of age and 3 months of age, mice were group housed and left undisturbed and were given food on hopper. Mice were group caged, except for the fasting re-feeding study and the activity experiment at 12 months of age, in transparent polyethylene cages (35 cm × 12 cm × 12 cm). Cages were equipped with pine chip bedding and a polyvinyl chloride tube (5 cm diameter, 8 cm length). Food pellets (Purina Rodent Chow, no. 5001) were weighed prior to placement within the cage. Tap water was available ad libitum. Mice were housed under a 12/12 hour reversed light/dark cycle with lights off from 09:30-21:30. All testing was completed during the dark phase of the light-dark cycle. All test procedures were approved by the Dalhousie Committee on Animal Care. Mice were genotyped for the APP and PS1 transgenes using tissue samples from ear-punches, using PCR by Dr. Chris Sinal (Pharmacology Department, Dalhousie University).

2.3.2 Food Intake and Body Weight Measures

2.3.2.1 Cross-sectional Analysis of Food intake and body weight

In the cross-sectional study we had a total of 29 5xFAD and 20 WT female mice. Five 5xFAD and five WT mice died at 9 months of age, therefore 24 5xFAD and 15 WT mice were tested at 9 months of age and 24 5xFAD mice and 15 WT mice were tested at 12 months of age. Food intake and body weight measures were made daily during three types of food presentations. For the first 7 days mice were given solid food in the food hopper, mice were then given solid food in the cage for 7 days, and then mice were given mashed food for 7 days. We gave both solid and mashed food in order to determine if these mice had motor impairments that affected their ability to ingest food. Body weight (BW) was measured via an electronic scale (Ohaus, Maryland) in grams (+/- 0.01). Food

pellets (solid and mashed) were weighed as well, and the difference between the initial mass of the pellets and the mass of the pellets after 24 hours was calculated. This difference in weights of the pellets indicated amount of food intake (FI). The cages were thoroughly investigated to see if there were any small fragments of waste food produced from the pellets during eating. These fragments were added to the mass of the final pellet weight (or weight of pellet after 24 hours). Body weights of WT and 5xFAD mice were analyzed using a two-way between design ANOVA (2 x 2), with genotype as a between subject factor and age as a within subject factor. T-tests were used to identify at what age differences were found in food intake and body weight. To correct for inflated type-1 error (false positives) we used the Bonferroni correction to counteract issues with multiple t-tests. Graphpad prism and Microsoft Excel 2008 were used to analyze data. The significance of statistical tests was set at $p< 0.025$.

2.3.2.2 Longitudinal Analysis of Food intake and body weight

In a longitudinal study 15 5xFAD mice and 15 WT mice were tested at 3 months of age, 14 5xFAD mice and 14 WT mice were tested at 6 months of age, 13 5xFAD mice and 13 WT mice were tested at 9 months of age, and 12 5xFAD mice and 13 WT mice were tested at 12 months of age. These mice were measured at 3, 6, 9, and 12 months of age for food intake and body weight. Refer to section 2.3.2.1 for protocol of experiment. Body weights of WT and 5xFAD mice were analyzed using a two-way mixed design ANOVA (2 x 4), with genotype as a between subject factor and age as a within subject factor. Analyses were made at 3, 6, 9, and 12 months of age. We used a 2-way repeated measures ANOVA for the longitudinal study measuring food intake and body weight. Because 3 5xFAD mice and 2 WT mice died during testing, we were forced to exclude them from analysis. T-tests were used to identify at what age differences were found in food intake and body weight. To correct for inflated type-1 error (false positives) we used the Bonferroni correction to counteract issues with multiple t-tests. Graphpad prism and Microsoft Excel 2008 were used to analyze data. The significance of statistical tests was set at $p< 0.0125$.

2.3.3 Fasting re-feeding

At 12 months of age all mice, from both the cross-sectional and longitudinal studies (44 5xFAD mice and 35 mice, however 8 5xFAD mice and 7 WT mice died before 12 months of age) were fasted for a minimum of 8 hours. Mice were then given pre-weighed mashed food and food consumption and body weight were measured at 1, 2, 4, 8, and 24 hours post-fasting. Mice were later exposed to the same fasting re-feeding procedure except given solid food in the cage. This was done to reduce food spillage which occurred in the mashed food presentation. For the fasting and re-feeding measurements we used a 2 x 5 mixed design ANOVA with genotype as a between subject factor and time (1, 2, 4, 8, and 24 hours) as a within subject factor. T-tests were used to identify at which times differences were found in food intake and body weight. To correct for inflated type-1 error (false positives) we used the Bonferroni correction to counteract issues with multiple t-tests. Graphpad prism and Microsoft Excel 2008 were used to analyze data. The significance of statistical tests was set at $p< 0.01$.

2.3.4 Correlation between food intake and body weight

In the correlation analysis we used 44 5xFAD mice and 35 mice (however 8 5xFAD mice and 7 WT mice died before 12 months of age) at 12 months of age food intake and body weight data were correlated to analyze the strength of the relationship between food consumption and body weight. We analyzed six linear correlations, each based on a separate food presentation (FOH, FIC, and M) and whether mice were WT or 5xFAD. Graphpad prism and Microsoft Excel 2008 were used to analyze data and compute Pearson's r and R^2. The significance of statistical tests was set at $p< 0.05$.

2.3.5 Activity Measures

Forty-four 5xFAD mice and 35 mice (however 8 5xFAD mice and 7 WT mice died before 12 months of age) were recorded for motor behaviour at 12 months of age. The behaviour of mice in their home cages was recorded using the computer program *Hindsight* event recording software (MS-Dos, version 1.5, Scott Weiss) for 20 minutes at 12 months of age to investigate the frequency of the following motor behaviours: grooming, rearing, jumping, climbing, and freezing.

2.3.6 Frailty Scores

Mice at 12 months of age (8 5xFAD mice and 6 WT) were evaluated with a non-invasive mouse frailty index test. This test consisted of 31 items that measured deficit accumulations detailed in the mouse frailty assessment form (Table 1; Whitehead et al., 2014). At 12 months of age, mice were exposed to food on hopper for 7 days, solid food in cage for 7 days, and mashed food for 7 days. At the end of each of those seven days, mice were given the frailty test. Clinical assessment included integument, musculoskeletal system, vestibulocochlear, auditory, ocular, nasal, digestive, urogenital system, respiratory, signs of discomfort, body weight (grams) and body temperature (degrees Celsius) ratings. We used the 12 month old WT mice with food on hopper as our baseline for body weight (grams) and body temperature (degrees Celsius). This was our selected control as younger mice do not reach plateau body weight until 9 to 12 months of age and the normal feeding method is to give mice food in the hopper. A score of 0 indicated no frailty, a score of 0.5 was given if there was mild frailty, a score of 1 indicated severe frailty. The frailty index gave a final score between 0-1. For frailty we used a one-way ANOVA to measure whether the WT or 5xFAD improved across food presentations and several t-tests to compare genotype. To correct for inflated type-1 error (false positives) we used the Bonferroni correction to counteract issues with multiple t-tests. Graphpad prism and Microsoft Excel 2008 were used to analyze data. The significance of statistical tests was set at $p< 0.017$.

Table 1 Mouse Frailty Assessment form. An index to measure the severity of disabilities in mice based on scoring parameters related to integument, musculoskeletal system, vestibulocochlear, auditory, ocular, nasal, digestive, urogenital system, respiratory, and discomfort ratings. Scores range from 0 to 1 for each parameter, with higher scores indicating higher levels of frailty than lower scores. (From Whitehead et al., 2014).

Date: _____

Mouse #: _____ **Date of Birth:** _____ **Sex:** F M

Body weight (g) _____ **Surface body temperature (°C)** _____

Mouse Frailty Assessment Form

Rating: 0 = absent 0.5 = mild 1 = severe

➢ **Integument:**					**NOTES:**
❖ Alopecia (hair loss)	0	0.5	1	_____
❖ Loss of fur colour	0	0.5	1	_____
❖ Dermatitis	0	0.5	1	_____
❖ Loss of whiskers	0	0.5	1	_____
❖ Coat condition	0	0.5	1	_____
➢ **Musculoskeletal system:**				
❖ Tumours	0	0.5	1	_____
❖ Distended abdomen	0	0.5	1	_____
❖ Kyphosis/hunched posture	0	0.5	1	_____
❖ Tail stiffening	0	0.5	1	_____
❖ Gait	0	0.5	1	_____
❖ Tremor	0	0.5	1	_____
❖ Forelimb grip strength	0	0.5	1	_____
❖ Body condition score	0	0.5	1	_____
➢ **Vestibulocochlear/Auditory:**				
❖ Head tilt	0	0.5	1	_____
❖ Hearing loss	0	0.5	1	_____
➢ **Ocular/Nasal:**				
❖ Cataracts	0	0.5	1	_____
❖ Discharge/swollen/ squinting	0	0.5	1	_____
❖ Microphthalmia	0	0.5	1	_____
❖ Corneal opacity	0	0.5	1	_____
❖ Vision loss	0	0.5	1	_____
❖ Menace reflex	0	0.5	1	_____
❖ Nasal discharge	0	0.5	1	_____
➢ **Digestive/Urogenital system:**				
❖ Malocclusions	0	0.5	1	_____
❖ Rectal prolapse	0	0.5	1	_____
❖ Penile/Uterine prolapse	0	0.5	1	_____
❖ Diarrhoea	0	0.5	1	_____
➢ **Respiratory:**				
❖ Breathing rate/depth	0	0.5	1	_____
➢ **Discomfort:**				
❖ Mouse Grimace Scale	0	0.5	1	_____
❖ Piloerection	0	0.5	1	_____

Total Score / Max Score:

2.4 RESULTS

2.4.1 Cross-sectional analysis of food intake and body weight

A two-way ANOVA was used to analyze food intake and body weight in our cross-sectional study. Twenty-nine 5xFAD and 20 WT female mice were used in the cross-sectional design. Five 5xFAD and five WT mice died at 9 months of age, therefore 24 5xFAD and 15 WT mice were tested at 9 months of age and 24 5xFAD mice and 15 WT mice were tested at 12 months of age. Although WT mice ate more than 5xFAD mice at both 9 and 12 months of age, there were no significant differences in food intake due to genotype (F (1, 35) < 1.00), or age (F (1, 35) < 1.00), and no genotype by age interaction (F (1, 35) < 1.00) when food was place in the hopper (Figure 11A).

Wild-type mice ate more than 5xFAD mice at 9 and 12 months of age, however there were no significant differences in food intake due to genotype (F (1, 35) = 1.123), or age (F (1, 35) < 1.00), and no genotype by age interaction (F (1, 35) <1.00) when food was placed in the cage (Figure 11B).

We did find a significant difference in food intake due to genotype (F (1, 35) = 9.464, p=0.0034) when food was mashed (Figure 11C). But there were no significant differences in food intake due to age (F (1, 35) <1.00), and no genotype by age interaction (F (1, 35) <1.00). T-tests showed that food intake differed in 9 month old mice (t= 2.432, df= 38, p=0.0204) but not in 12 month old mice (t= 1.344, df = 38, p= 0.1921).

WT mice weighed more than 5xFAD mice whether food was presented in the hopper (F (1, 35) = 47.16, p< 0.0001; Figure 12A), in the cage (F (1, 35) = 40.19, p<0.0001; Figure 12B), or as mashed food (F (1, 35) = 32.26, p<0.0001; Figure 12C). At 9 months of age WT weighed more than 5xFAD mice whether food was presented in the hopper (t = 3.810, df = 38, p = 0.0006; Figure 12A), in the cage (t = 3.371, df = 38, p = 0.0019; Figure 12B), or as mashed food (t = 3.012, df = 38, p = 0.0049; Figure 12C). At 12 months of age WT weighed more than 5xFAD mice whether food was presented in the hopper (t = 5.773, df = 38, p <0.0001; Figure 12A), in the cage (t = 5.457, df = 38, p <0.0001; Figure 12B), or as mashed food (t = 4.93, df = 38, p <0.0001; Figure 12C). Overall, 5xFAD mice exhibited a 15% decrease in body weight at 9 months of age and a 21% decrease in body weight at 12 months of age.

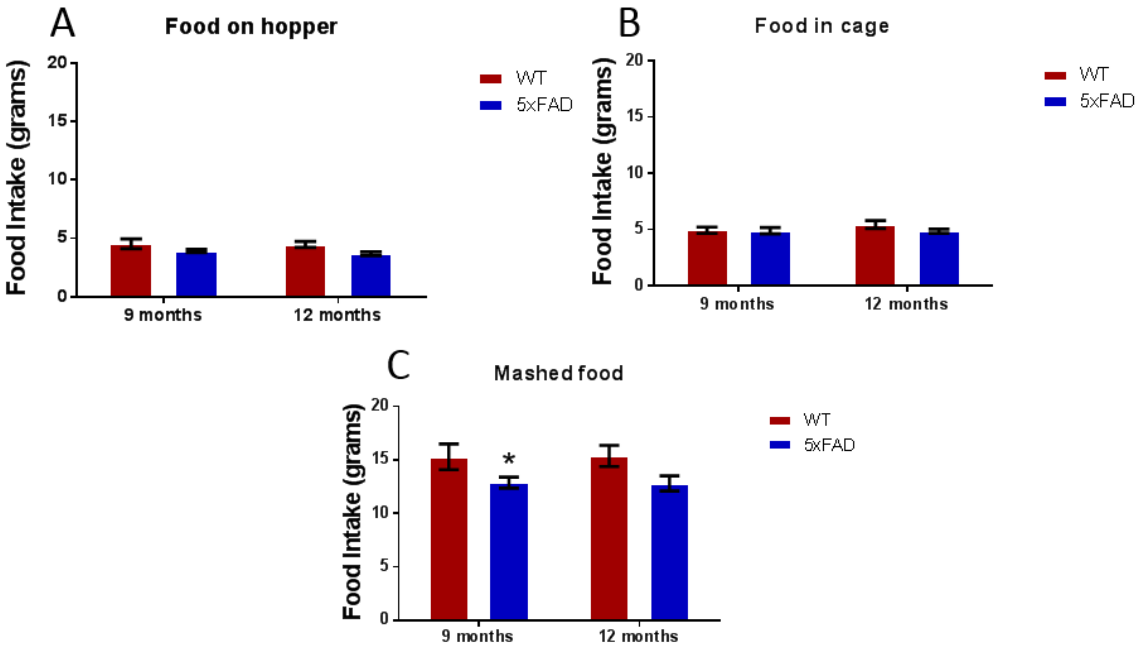

Figure 11 Analysis of food consumption. The weight of food consumed was analyzed in our cross-sectional design at 9 and 12 months of age under different food presentations. No genotype differences were seen when food was on the hopper (A) or in the cage (B). However, WT mice ate more mashed food than 5xFAD mice at both ages (C) but this difference was only significant at 9 months of age ($p<0.025$). * $p<0.025$

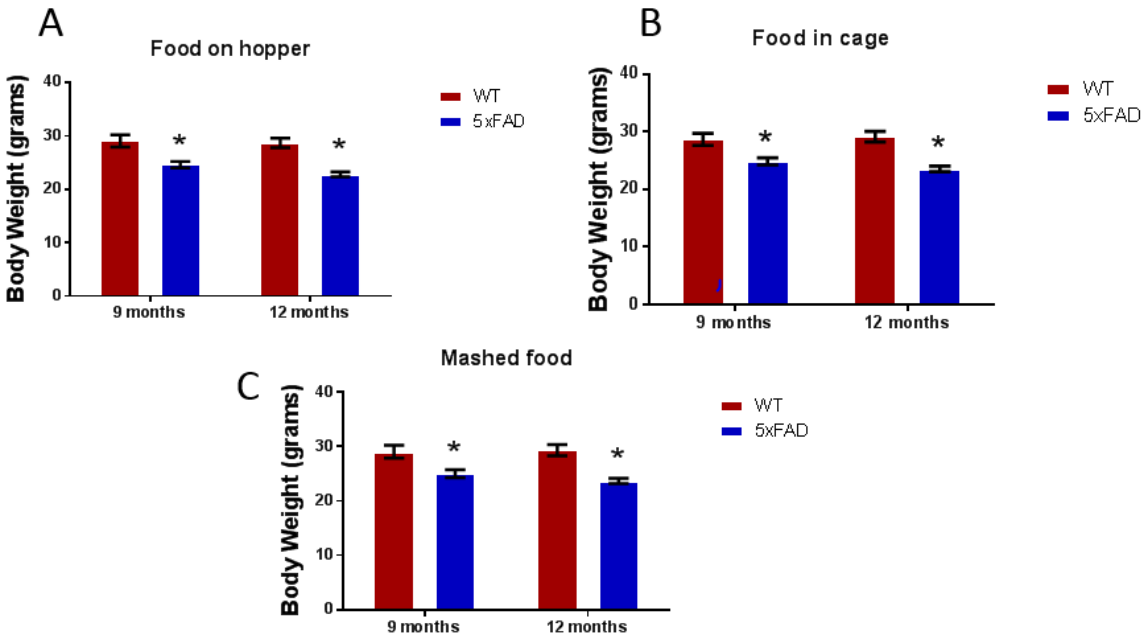

Figure 12 Analysis of body weight in cross-sectional mice at 9 and 12 months of age. WT mice weighed more than 5xFAD mice at both ages and under all three different food conditions: food on hopper (A), food in cage (B), and mashed food (C). * ($p < 0.025$).

2.4.2 Longitudinal study of food intake and body weight

With the longitudinal study we did several 2-way repeated measures ANOVAs. We used 15 5xFAD and 15 WT female mice, however 3 5xFAD and 2 WT mice died during the 12 month measures, and we were forced to exclude them from analysis. We found significant differences in food intake due to genotype (F (1, 17) = 13.66, p = 0.0012), age (F (3, 17) = 10.26, p< 0.0001), and genotype by age interaction (F (3, 17) = 10.73, p <0.0001; Figure 13A). When food was presented on the hopper the WT ate more than the 5xFAD mice ate at each age but these differences were significant only at 9 months (t= 2.832, df= 24, p=0.0097) and 12 months (t= 4.342, df=24, p=0.0002) of age (Figure 13A).

When food was presented in the cage we found significant differences in food intake due to genotype (F (1, 17) = 3.917, p = 0.043) and age (F (3, 17) = 4.871, p= 0.0039; Figure 13B), but no significant genotype by age interaction (F (3, 17) =1.659, p=0.1838). Wild-type mice ate more than 5xFAD mice at each age but these differences were significant only at 9 months (t= 4.555, df = 24, p= 0.0002) and 12 months (t=3.232, df=24, p= 0.0037) of age (Figure 13B).

When food was presented as mashed food we found a significant difference in food intake due to age (F (3, 17) = 5.474, p= 0.002) as mice ate less as they got older. But there were no significant differences in food intake due to genotype (F (1, 17) <1.00, p =0.3558) and no significant genotype by age interaction (F (3, 17) = 1.373, p = 0.2583; Figure 13C).

Wild-type mice weighed more than 5xFAD mice whether food was presented in the hopper (F (1, 17) = 8.673 p = 0.0073; Figure 14A), in the cage (F (1, 17) = 8.673, p = 0.0073; Figure 14B), or as mashed food (F (1, 17) = 8.75, p = 0.0071; Figure 14C). When food was presented in the hopper WT weighed more than 5xFAD mice at each age but these differences were only significant at 9 months (t = 3.104, df = 24, p = 0.0052) and 12 months (t = 3.897, df = 24, p = 0.0007) of age (Figure 14A). When food was presented in the cage, WT weighed more than 5xFAD mice at each age but these differences were only significant at 9 months (t = 2.938, df= 24, p = 0.0076) and 12 months (t = 3.525, df = 24, p = 0.0018) of age (Figure 14B). Wild-type mice weighed more than 5xFAD mice at each age under the mashed food presentation, but these differences were significant only

at 9 months (t = 3.183, df = 24, p = 0.0043) and 12 months (t =3.581, df = 24, p = 0.0016) of age (Figure 14C)

There were significant differences in weight due to age whether food was presented in the hopper (F (3, 17) = 32.08 p< 0.0001), in the cage (F (3, 17) = 27.95, p<0.0001), or as mashed food (F (3, 17) = 28.35, p< 0.0001). We also found significant differences in weight due to genotype by age interaction whether food was presented in the hopper (F (3, 17) = 11.35, p<0.0001), in the cage (F (3, 17) = 9.823 p< 0.0001), or as mashed food (F (3, 17) = 9.384, p< 0.0001).

They were the same mice, weighed one week apart. Both genotypes gained weight with age, resulting in a significant age effect, but WT gained more weight than 5xFAD mice as they aged, resulting in a significant age by genotype interaction. WT were significantly heavier than 5xFAD mice at 9 and 12 months of age. The different feeding methods did not change these effects.

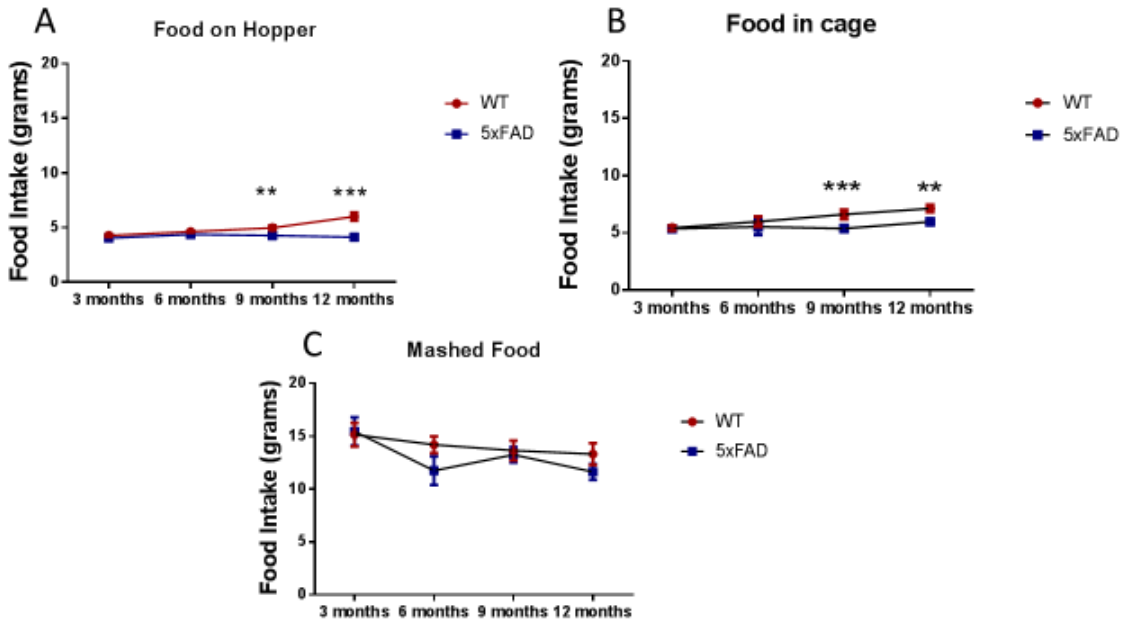

Figure 13 Analyzing food intake at 3, 6, 9, and 12 months of age revealed that differences in food consumption occurred at 9 and 12 months of age when food was presented on the hopper and in the cage. In both cases WT ate more than the 5xFAD. *p<0.0125, **p<0.01, ***p<0.001

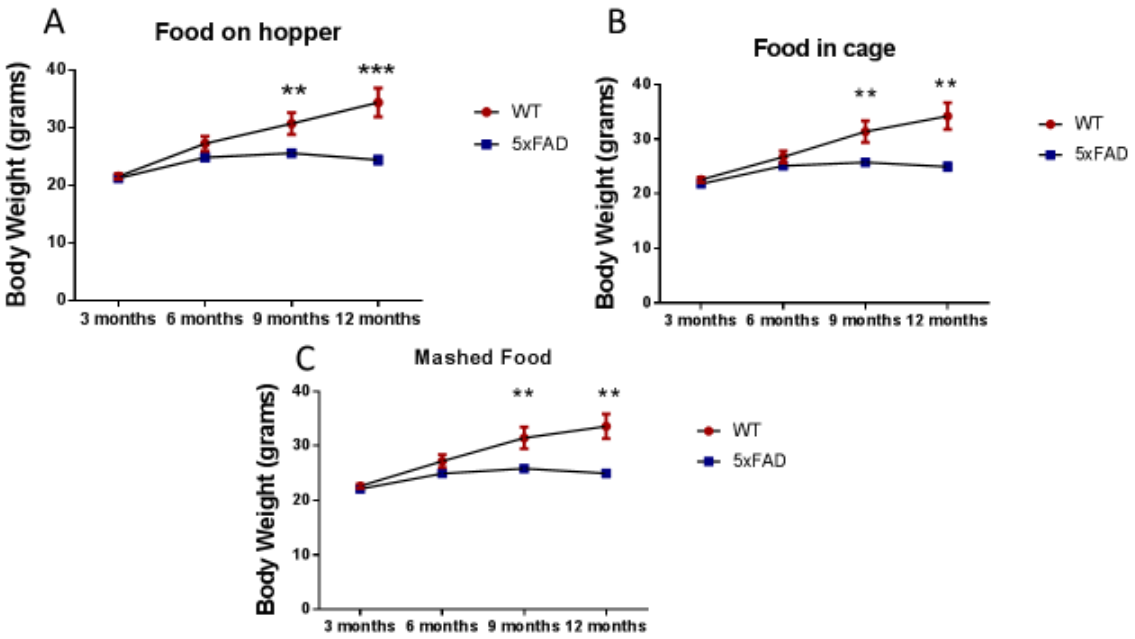

Figure 14 Measuring body-weight at 3, 6, 9, and 12 months of age under different food presentations found that in all food conditions 5xFAD mice weighed less than WT at 9 and 12 months of age.

2.4.3 Fasting re-feeding: an evaluation of food intake and body weight over 24 hours

A two-way ANOVA was done to measure food intake and body weight over 24 hours when solid or mashed food was provided. We used 44 5xFAD and 35 WT female mice, however 8 5xFAD mice and 7 WT mice died before 12 months of age. Mice were measured at 12 months of age. There were significant differences in food intake due to genotype (F (1, 54) = 171.6, p = <0.0001) and hours (F (4, 54) = 3.319, p = 0.0065) when food was presented in the cage (Figure 15A). Differences in food intake were significant at 24 hours (t =2.982, df = 63, p = 0.0053) post fasting for solid food intake (Figure 15A).

There was a significant difference in body weight due to genotype (F (1, 52) = 108.1, p <0.0001; Figure 15B). Wild-type mice weighed significantly more than 5xFAD mice at all time intervals (p<0.008; Figure 15B).

When mash was provided, there were significant differences in food intake due to genotype (F (1, 54) = 23.97, p <0.0001) and time (F (4, 54) = 124.3 p < 0.0001; Figure 15C). Differences in food intake were significant at each time point: 1 hour (t = 3.341, df = 63, p = 0.002), 2 hrs (t = 3.161, df = 63, p = 0.0031), and 24 hrs (t=3.241, df= 63, p = 0.0024) hours post fasting (Figure 15C). No significant differences were seen at 4 hrs (t= 2.099, df= 63, p=0.043) and 8 hours (t=1.544, df= 63, p=0.13807).

When mashed food was presented we found significant differences in body weight due to genotype (F (1, 52) = 9.45, p = 0.0025), age (F (5, 52) = 223.3, p <0.0001), and a genotype by age interaction (F (5, 52) = 5.052, p = 0.0007; Figure 15D). Wild-type mice weighed more than 5xFAD mice at all time intervals (p<0.008; Figure 15D) and gained more weight than 5xFAD mice.

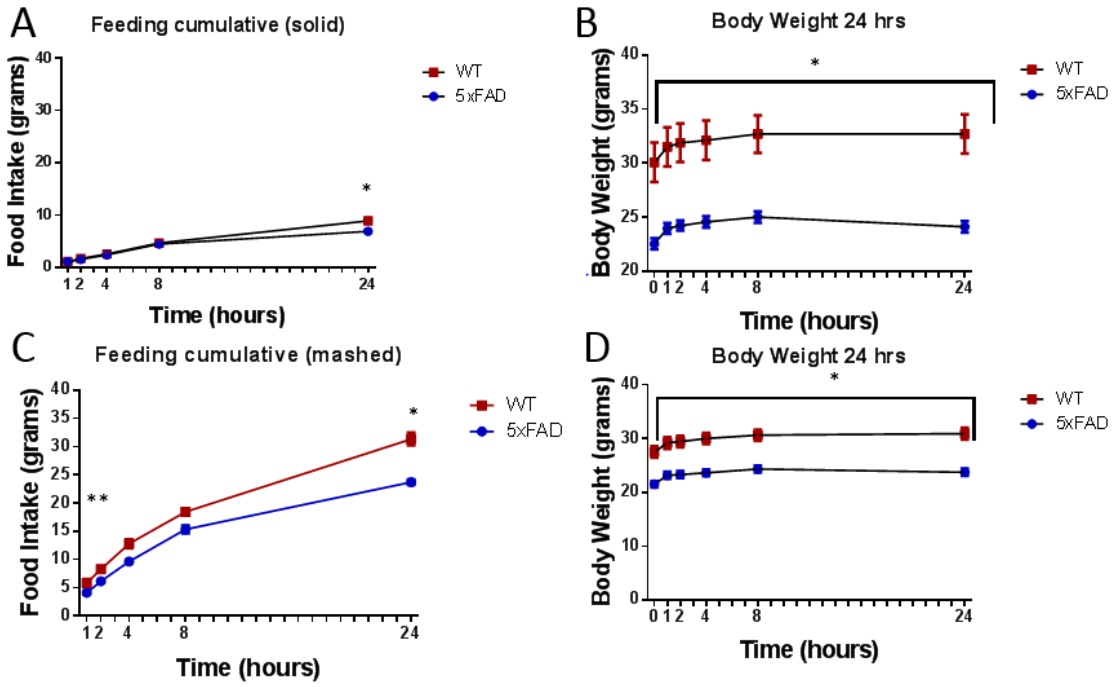

Figure 15 Measures of food intake and body weight were completed over 24 hours after fasting. Whether solid or mashed food was presented in the cage, WT mice ate and weighed more than the 5xFAD mice ($p<0.008$).

2.4.4 Correlation between food intake over 24 hours and body weight

All mice were used in this experiment, 44 5xFAD and 35 WT female mice, however 8 5xFAD mice and 7 WT mice died before 12 months of age. Measurements were taken from 12 month old mice. Analysis of correlations between food intake over 24 hours and body weight revealed that 5xFAD mice, when food was presented on the hopper, had a Pearson's r= 0.2036, $df = 63$, $p = 0.2988$, and $R^2 = 0.04145$ (Figure 16A). When the 5xFAD mice were exposed to food inside the cage the Pearson's r = 0.05276, $df = 63$, $p = 0.7898$, and $R^2 = 0.002783$ (Figure 16B). The 5xFAD had a Pearson's r = -0.5466, $df = 63$, $p = 0.0026$, and a $R^2 = 0.2987$ when food was mashed (Figure 16C). The negative correlation may be due to food spillage.

Wild-type mice had a Pearson's r = 0.5733, $df = 63$, $p = 0.0014$, and $R^2 = 0.3287$ when food was placed on the hopper (Figure 17A). When food was placed in the cage WT mice had a Pearson's r = 0.5014, $df = 63$, $p = 0.0066$, and $R^2 = 0.2514$ (Figure 17B). When food was mashed WT mice had a Pearson's r = -0.3801, $df = 63$, $p = 0.046$, and $R^2 = 0.1445$ (Figure 17C). This negative correlation may be due to food spillage.

Correlations were not significant between body weight and food intake for 5xFAD mice except when food was placed in the hopper. Correlations were significant between body weight and food intake for WT mice.

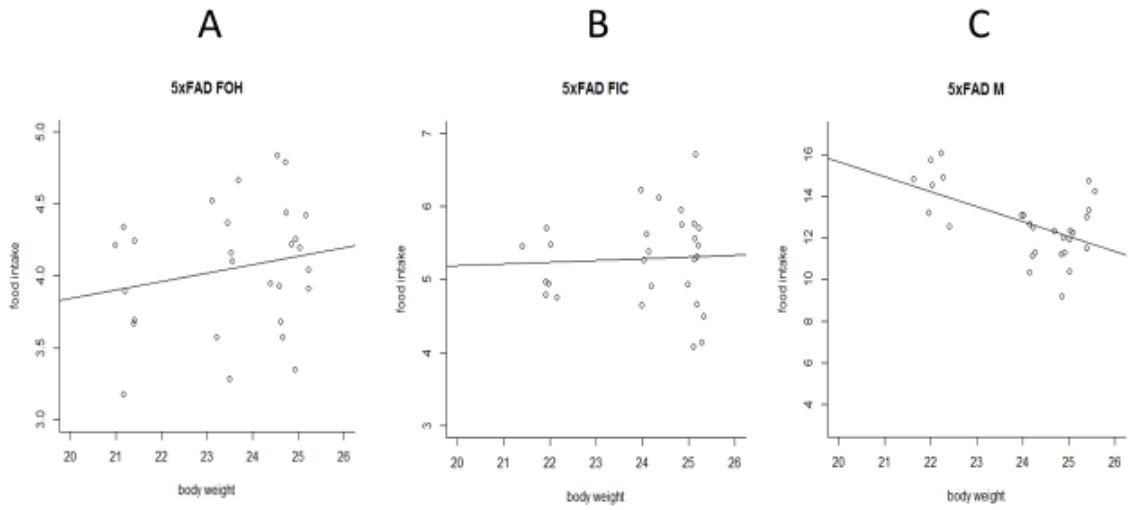

Figure 16 Correlation between food intake and body weight in 12 months 5xFAD mice. The 5xFAD had a Pearson's r = -0.2036, *df*= 63, *p* = 0.2988, and a R^2 = 0.04145 when food was on the hopper (A). When food was presented in the cage, the 5xFAD mice had a Pearson's r = 0.05276, *df* = 63, *p* = 0.7898, and R^2 = 0.002783. The 5xFAD mice had a Pearson's r = -0.5466, *df* = 63, *p* = 0.0026, and a R^2 = 0.2987. FOH = food on hopper, FIC = food in cage, and M= mashed food.

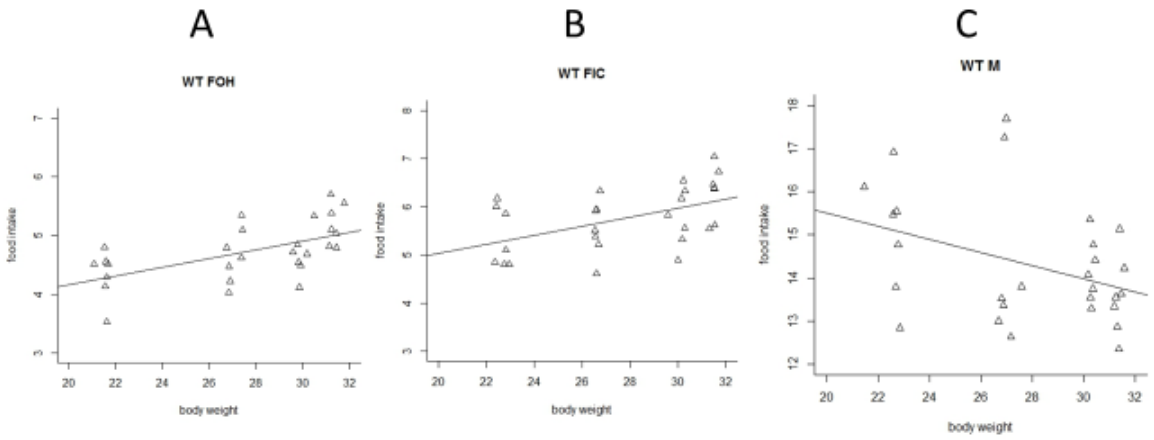

Figure 17 Correlation between food intake and body weight in WT mice. Wild-type mice had a Pearson's r = 0.5733, *df*= 63, *p*= 0.0014, and R^2 = 0.3287 when food was placed on the hopper. When food was placed in the cage WT mice had a Pearson's r = 0.5014, *df*= 63, *p*= 0.0066, and R^2= 0.2514. When food was mashed WT mice had a Pearson's r = -0.3801, *df*= 63, *p*=0.046, and R^2 = 0.1445. FOH = food on hopper, FIC = food in cage, M = mashed food.

2.4.5 Activity measures

All mice were used in this experiment, 44 5xFAD and 35 WT female mice, however 8 5xFAD mice and 7 WT mice died before 12 months of age. T-tests were done to analyze genotype differences in frequency of rearing, climbing, freezing, grooming, and jumping in the home cage observation. Wild-type mice exhibited more bouts of rearing ($t = 4.861$, $df = 63$, $p < 0.0001$; Figure 18A), and climbing ($t = 3.327$, $df = 63$, $p = 0.0031$; Figure 18B) than 5xFAD mice. The 5xFAD mice showed more freezing/inactivity behaviour than WT mice ($t = 2.69$, $df = 63$, $p = 0.0133$; Figure 18C). No differences were seen for grooming or jumping ($p>0.05$; Figure 18D and 18E).

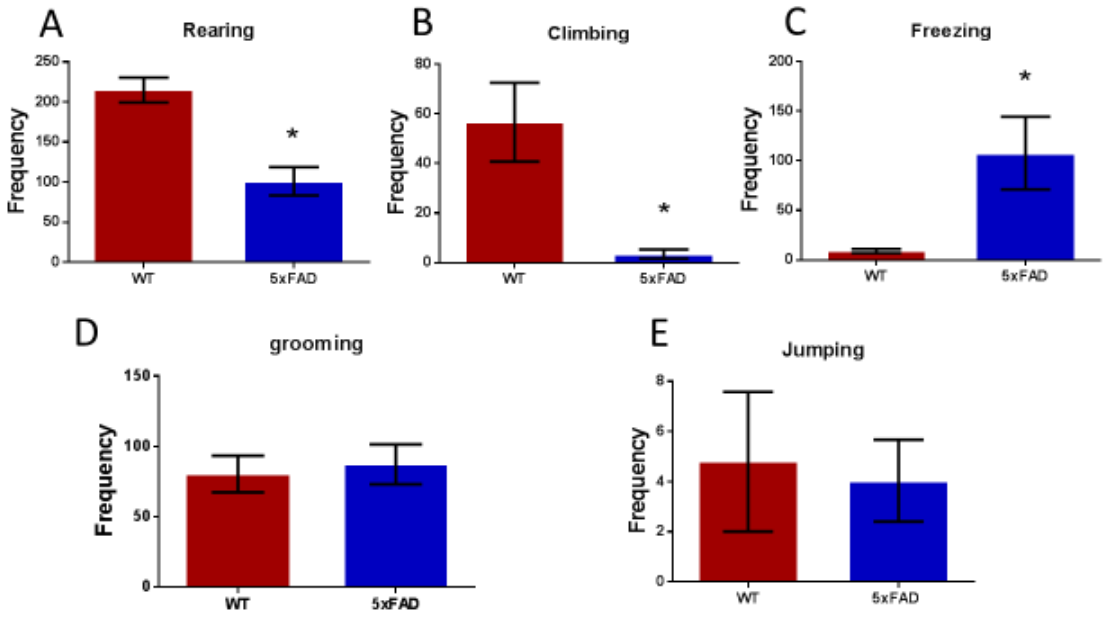

Figure 18 Frequency of jumping, freezing, climbing, grooming, and rearing were analyzed. Differences were seen in freezing, climbing, and rearing. WT spent more time rearing and climbing compared to the 5xFAD, whereas 5xFAD exhibited frequency more frequently than the WT ($p<0.05$).

2.4.6 Frailty Scores

Eight 5xFAD and 6 WT female mice at 12 months of age were used in this experiment. A one-way ANOVA followed by t-tests were used to analyze frailty scores between the 5xFAD and WT mice. A Bonferroni correction test was used to reduce type-1 error inflation. A significant difference was found in the food on hopper condition (t = 3.923, df = 13, p = 0.0021) where 5xFAD scored higher on frailty than WT (Figure 19). Although no significant differences (p>0.017) were seen in frailty when food was in the cage or mashed, we did see that 5xFAD mice had a higher frailty score than the WT in the FIC and M presentation. Neither WT nor 5xFAD mice differed in frailty scores across all food presentations (p>0.017).

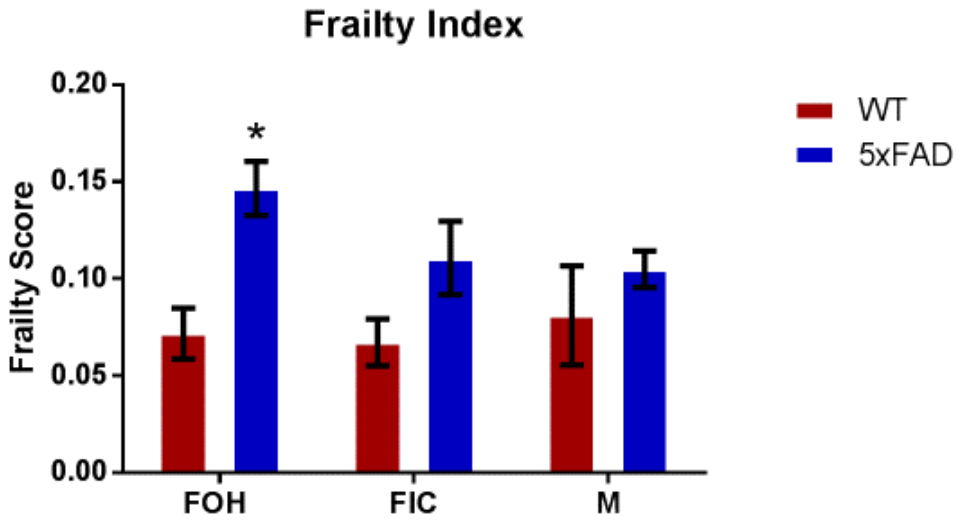

Figure 19 Frailty scores were analyzed amongst and between genotypes. A significant difference was seen when food was presented on the hopper ($p<0.017$). WT mice showed lowered frailty scores than WT in all food presentations. FOH = food on hopper, FIC = food in cage, M = mashed food.

2.5 DISCUSSION

2.5.1 Why did we do this study?

This topic was investigated because we observed weight loss and motor dysfunction in 5xFAD mice (O'Leary, 2013). We hypothesized that their weight loss might be due to their inability to obtain food from the hopper. We therefore put the food pellets on the cage floor and offered them mash to see if different types of food presentations increased body weight.

2.5.2 5xFAD show reduction in food intake when solid but not mashed food is presented

When measuring food intake we found that in both the cross-sectional and longitudinal design food intake was reduced at 9 and 12 months of age in 5xFAD mice when food was on the hopper and in the cage. In both cases WT ate more than the 5xFAD. When food was mashed we found no significant genotype differences, however this result may be confounded by food spillage when food was presented as mash. Even within the fasting and re-feeding condition, WT mice ate significantly more than 5xFAD mice over 24 hours. The results show that beginning at 9 months of age 5xFAD mice eat less solid food than WT controls. Thus the type of food given (solid vs mash) may correlate to weight loss in 5xFAD mice.

2.5.3 5xFAD mice exhibit reduced body weight gain at 9 and 12 months of age

In all food presentations and during the fasting re-feeding experiment, 5xFAD mice showed lower body weight than WT mice beginning at 9 months of age. Indeed, it made no difference whether food was placed in the cage, or mashed, lack of weight gain occurred in the 5xFAD mice. One week of food in the cage or mashed food may not be long enough to alter body weight and a longer or permanent exposure to these food conditions might result in increased body weight in 5xFAD mice.

2.5.4 Correlation between food intake and body weight is high for WT but not for 5xFAD mice

In WT mice, there was a significant relationship between food intake and body weight when food was on the hopper or in the cage: larger mice ate more food. We found a negative correlation for WT mice when food was mashed (-0.38). This is most likely because of food spillage. Consequently, this leads to food spillage and an overestimation in food consumption. This was also seen in the 5xFAD mice (-0.5). When we ran correlations for the 5xFAD mice between food intake and body weight, there was no significant relationship when food was presented on the hopper and in the cage, however this may indicate that larger mice do not eat more food in the 5xFAD mice as occurs in the WT. These results suggest that there are factors other than food intake that influence body weight in the 5xFAD mice.

2.5.5 Hypoactivity is seen in the 5xFAD mice

The 5xFAD mice exhibited less rearing and climbing than WT mice and the 5xFAD mice spent more time being inactive or still than their WT counterparts. From these observations, it appears that the 5xFAD mice exhibited fewer motor behaviours than WT mice, especially when these behaviours required much exertion. There were no genotype differences in the frequency of grooming or jumping but for jumping we saw a large degree of variability in both genotypes, which may have affected our results and interpretations.

2.5.6 Frailty scores are higher in 5xFAD mice

The 5xFAD mice had a higher frailty score than WT mice, especially when food was presented on the hopper. Although we saw no significant differences in frailty when food was presented in the cage or mashed, 5xFAD mice scored higher on frailty than WT. We also found that food presentation did not significantly influence frailty scores in either genotype. However, perhaps one week of exposure to food in cage or mashed food may not be long enough to have an effect on frailty. Furthermore, frailty scores are designated as either 0, 0.5, or 1. This scale is not very flexible and may lead to an underestimation of frailty found in our 5xFAD mice.

2.5.7 General Discussion

The present study measured food intake and body weight at 3, 6, 9, and 12 months of age under different food presentations. We found that food intake and body weight were reduced in 5xFAD mice beginning at 9 months of age, continuing to 12 months of age. Jawhar et al. (2010) also found weight reductions in their 5xFAD mice beginning at 9 months of age. This supports the finding that 5xFAD mice display progressive weight loss as seen in AD patients (Barrett-Connor et al., 1996). Furthermore, 5xFAD mice also display a reduction in food intake also seen in a subset of patients with AD (Sergi et al., 2013). We also noted that our 5xFAD mice were not as active as their WT counterparts. This led us to believe these mice are hypoactive. Furthermore, the 5xFAD mice exhibited freezing more frequently. This may be a sign of catatonia, also seen in AD patients at the later stages of the disease (Jerry, 2006). Although food intake is reduced at 9 and 12 months of age in the 5xFAD mice, this reduction of food only partially explains the weight loss seen in the 5xFAD mice, as the strength of the relationship between food intake and body weight was weak. Frailty was elevated in 5xFAD mice and that different food presentations did not ameliorate frailty in either genotype. Due to reductions in food intake in all food presentations for the 5xFAD mice, motor deficits in these mice do not appear to influence their food consumption. If motor impairments did affect food consumption, we would expect to see the highest difference with food on the hopper and no difference in food consumption between genotypes when food became more accessible. The 5xFAD mice exhibited reduced motor behaviours such as rearing and climbing, yet spent more time remaining still or being inactive. Consequently, the 5xFAD mice are not hyperactive indicating that hyperactivity does not contribute to weight loss seen in the 5xFAD mice. Frailty scores in the 5xFAD mice were higher than WT mice suggesting that the 5xFAD have more deficits in behaviours and body condition such as vision and fur quality. None of the food presentations reduced frailty scores seen in the 5xFAD mice.

Potential factors that may explain the weight loss but have yet to be evaluated in this mouse model are hypermetabolism, malabsorption of nutrients (an inability to absorb sugars, vitamins, fats, or proteins), and the sick state (a state in which feeding is reduced in response to illness). Although hypermetabolism has yet to be concretely supported in

AD patients, some mouse models of AD exhibit hypermetabolism such as the 3xTg-AD mouse (Knight et al., 2010; Niskamen et al., 1993; Poehlman and Dvorak, 2000; Sancheti et al., 2014). The 3xTg-AD mice consumed more oxygen (24%) and produced more carbon dioxide (29%) than their WT counterparts, suggesting that the 3x-TgAD were in an elevated metabolic state (Knight et al., 2010). No study has yet to evaluate the metabolic state of the 5xFAD mice. Patients with AD show no sign of malabsorption of nutrients although they show deficiency in nutrients and fatty acids (Sergi et al., 2013). Lack of these nutrients may be due to reduced food intake and poor diet (i.e. lack of important vitamins such as B_{12}, C, and D; Shatenstein et al., 2007). Reduced levels of important nutrients such as vitamin B_{12} may also exacerbate cognitive symptoms (Singh et al., 1988). No study has investigated whether 5xFAD mice exhibit reduced levels of nutrients or experience malabsorbtion. For the sick state, patients who are afflicted with a cold or flu exhibit sickness behaviour which includes reduced food intake and body weight loss (Dantzer et al., 2008). This is likely due to the secretion of pro-inflammatory cytokines such as TNF-α and IL-6 which regulate feeding centers (i.e. hypothalamus) in the brains and suppress food intake. The AD brain is in a neuroinflammatory state. This is because regions in the brain that contain Aβ plaques are bombarded with microglia that secrete pro-inflammatory cytokines such as TNF-α and IL-6 (Sergi et al., 2013). These regions include the medial temporal cortex, anterior cingulate cortex, both of which are involved in the regulation of food intake and body weight by the hypothalamus (Sergi et al., 2013). Consequently, food intake and body weight may be reduced because of elevated levels of TNF-α and IL-6 in the 5xFAD mice, and these cytokines should be examined in the 5xFAD mouse model.

Limitations of this project involve the mashed food and the exposure to food presentations. For mashed food, food spillage was always an issue and food intake may have been overestimated, especially with the mashed food. Exposure to food presentations was problematic in that mice were generally exposed to food on hopper for the majority of their lifespan and given food in hopper and mashed food for one week each. Consequently, lack of effect for food presentation for either body weight or frailty scores may be due to brief exposure to food presentations.

2.5.8 Conclusion

5xFAD mice exhibited reductions in food intake at 9 and 12 months of age, particularly when food was presented in hopper and in the cage. Body weight reduction was also seen at 9 and 12 months of age in the 5xFAD mice. Food reduction in 5xFAD likely only partially explains for weight loss as the relationship between food intake and body in 5xFAD mice is weak. Frailty was elevated in 5xFAD compared to WT mice and different food presentations appeared to have no effect on frailty.

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CHAPTER 3 NEUROENDOCRINE MECHANISMS UNDERLYING AGE-RELATED WEIGHT LOSS IN 5XFAD MICE

3.1 ABSTRACT

The 5xFAD mouse is a double transgenic model of Alzheimer's disease (AD), which carries an amyloid precursor protein (APP) transgene with three mutations, and a presenilin-1 transgene with two mutations which produce increases in beta amyloid (Aβ)-peptides. The presence of AB-plaques in this mouse model emerges at two months of age. Age-related weight loss is seen at 12 months of age in 5xFAD mice and this paper investigated age-related changes in fat and muscle tissue, insulin, glucose, and mRNA expression of leptin, uncoupling protein-2 (UCP-2), hormone sensitive lipase (HSL), and uncoupling protein-1 (UCP-1) in 12 month old female 5xFAD mice and their WT (C57BL/6JxSJL/J F1) controls. Perigonadal white adipose tissue (WAT), interscapular brown adipose tissue (BAT) and the gastrocnemius muscle tissue were collected and it was found that 5xFAD mice had less WAT than WT mice ($p<0.05$). The 5xFAD mice did not differ from WT mice in BAT weight or in muscle mass ($p>0.05$). We collected blood at 12 months of age and found no genotype differences in plasma concentration of insulin or glucose ($p>0.05$). Expression of leptin mRNA, UCP-2, and HSL were measured in WAT. The 5xFAD mice expressed reduced levels of these mRNAs ($p<0.05$). Expression of UCP-1 mRNA was measured in BAT and 5xFAD mice expressed more UCP-1 than WT mice ($p<0.05$). Our data indicates that 5xFAD mice have reduced WAT, but not BAT or muscle mass compared to WT mice. Finally, weight loss due to elevated UCP-1 expression, may be attributed by a reduction in adipogenesis and adiposity, due to reduced leptin, UCP-2, and HSL, and an elevation in thermogenesis leading to reduced fat storage and increased fat burn.

3.2 INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disease that affects seniors over 60 (Minati et al., 2009). The majority of human dementia cases are due to Alzheimer's disease (Luchsinger et al., 2009). The prevalence of AD is expected to quadruple by the year 2047 (Luchsinger et al., 2009). Consequently, it is important to develop methods to detect AD before cognitive symptoms emerge and to develop therapeutic interventions that may prevent, cure, or alleviate symptoms of AD. Cognitive symptoms of AD include memory and language loss, and peripheral impairments, such as motor rigidity and weight loss (Devi et al., 2012; Fewlass et al., 2004). Factors that influence weight loss remain elusive (Gillete et al., 2007). AD patients tend to exhibit memory loss accompanied with weight loss, and this weight loss may precede the onset of memory dysfunction (Barrett-Connor et al., 1996). Weight loss reduces life expectancy and autonomy in AD patients (Adebakin et al., 2012; Hebert et al., 2010). Hence, additional research is warranted to determine the biological basis for alterations in body weight loss in AD.

Patients with AD exhibit reduced fat and muscle mass compared to non-AD aged seniors (Lowry, 2010; Renvall et al., 1993). This is seen in some mouse models of AD such as the Tg2576 (Ishii et al., 2014). This atrophy may be attributed to reduced leptin concentrations in blood plasma and an insulin resistant brain in AD patients (Lieb et al., 2009; Craft et al., 1998). Leptin and insulin are hormones that mediate appetite and body weight via their receptors on neurons within the arcuate nucleus of the hypothalamus (Barsh and Schwartz, 2002). The hormones are anorexigenic, therefore inhibiting NPY (neuropeptide Y) and Agrp (Agouti related peptide) neurons that promote food intake and activate CART (cocaine and amphetamine regulated transcript receptors) and POMC (proopiomelanocortin) neurons that promote satiation (Barsh and Shwartz, 2002). Leptin is produced from adipocytes and insulin is produced from β-cells of the pancreas.

Patients with AD tend to exhibit elevated insulin concentrations in the plasma and reduced insulin concentrations in the brain (Craft et al., 1998). No mouse model to date has been shown to express this phenotype. However, AD patients also have persistent phosphorylation on serine residues of insulin receptors (IRs), which lead to inhibition of insulin signaling (Bomfim et al., 2012). This is due to persistent activation of the c-Jun

N-terminal kinase (JNK)/ TNF-α pathway, which phosphorylates serine residues of IRS (Bomfim et al., 2012). Mouse models of AD (i.e. APP/PS1) exhibit this persistent activation of the JNK/TNF-α pathway, and therefore also show insulin resistance (Bomfim et al., 2012).

Although hypermetabolism has yet to be seen in AD patients, the 3x-TgAD mouse models of AD exhibits hypermetabolism (Poehlman and Dvorak, 2000; Knight et al., 2010). The 3x-TgAD consumed more oxygen (24%) and produced more carbon dioxide (29%) than their WT counterparts, supporting the hypothesis that 3x-TgAD mice are in an elevated metabolic state (Knight et al., 2010). One marker for hypermetabolism is uncoupling protein-1 (UCP-1). The inner mitochondrial membrane contains UCP-1, which is predominately found in brown adipose tissue (BAT; Yo et al., 2013). Elevated expression of UCP-1 has been associated with increased thermogenesis (converting adenosine triphosphate to heat in order to prevent hypothermia) and fat burn (Yo et al., 2013). Furthermore, humans and mice that have elevated metabolism exhibit elevated BAT and UCP-1 activation and expression (Yo et al., 2013). However, AD patients show no elevated expression of UCP-1 (Cornelius et al., 2013). Nonetheless, it is important to determine if UCP-1 is elevated in mouse models of AD that display hypermetabolism.

UCP-2, like UCP-1, is a mitochondrial protein found in the inner mitochondrial membrane (Vidal-Puig et al., 1997). This protein is expressed throughout the body but is predominantly expressed in white adipose tissue (Vidal-Puig et al., 1997). Its function is similar to UCP-1 in that it separates oxidative phosphorylation from adenosine triphosphate synthesis (Arsenijevic et al, 2000). This results in the production of heat. Consequently, UCP-2 also regulates thermogenesis (Arsenijevic et al, 2000). Furthermore, UCP-2 limits the production of reactive oxidative species (Arsenijevic et al, 2000). Indeed, knockout mice lacking UCP-2 display elevated concentrations of reactive oxidative species (Arsenijevic et al, 2000). UCP-2 is up-regulated by leptin (Zhang et al., 2008). This relationship indicates that if leptin levels are reduced, then expression of UCP-2 should be reduced. Hence, measuring UCP-2 will indicate indirectly if leptin expression is high or low.

Transgenic mouse models have been valuable in studying the molecular and behavioural processes that occur in AD (Shineman et al., 2001; Webster et al., 2014).

These mouse models are useful to assess the efficacy of proposed novel treatments for AD. Most of these models have been used to study cognitive deficits rather than weight-loss and other symptoms seen in AD. The double transgenic 5xFAD mouse model expresses similar age-related weight loss to that seen in patients with AD (see chapter 2; Jawhar et al., 2010). These mice have mutant human APP with three mutations (Sweden, London, Florida) and two PS1 mutations (M146L and L286V; Oakley et al., 2006). The net effect of these mutations is to increase the concentration of Aβ monomers that later oligomerize and induce toxic effects (Bomfim et al., 2012). The 5xFAD mouse is a reliable and valid mouse model of AD as AD symptoms develop more rapidly relative to other mouse models (Jawhar et al., 2010). 5xFAD mice are useful to study disease mechanisms underlying cognitive deficits and potentially weight-loss. No research to date has used 5xFAD mice to assess tissue atrophy or the metabolic hormones and proteins such as insulin, leptin, and UCP-1 concentrations and expression. No study has attempted to measure expression of hormone sensitive lipase (HSL), a marker for adipose catabolism found in white and brown adipose tissue (Zechner et al., 2014). HSL regulates free fatty acid secretion into the circulation (Haemmerle et al., 2001). Consequently, constant activation of HSL leads to leaner body types (Cummings et al., 1996).

The aims of this study were first to evaluate white and brown adipose tissue mass in 5xFAD mice and to measure the gastrocnemius muscle to investigate if this mouse model experiences sarcopenia. We then investigated insulin and glucose concentrations in the plasma. Finally, we investigated the expression of leptin, HSL, and UCP-2 mRNA in WAT and UCP-1 mRNA expression in BAT.

3.3 Methods

3.3.1 Animals

Twenty-one 5xFAD and 26 WT female mice were used in this study. Male 5xFAD were purchased from Jackson labs (model number: B6SJLT-Tg (APPSwFlLon, PSEN1*M146L*L286V) 6799Vas/Mmjax), and bred with female wild-type C57BL/6JxSJL/J F1 mice and the F2 offspring were used in the present study. Breeding occurred in the Brown lab. All mice were female because majority of AD patients are

women. Consequently, we were interested as to what contributed to women's susceptibility to the disease. These mice had been given behavioural tests at 3, 6, 9, and 12 months of age (see chapter 2), and the tissue from these mice were taken after 12 months of age because significant weight discrepancy between WT and 5xFAD occurs generally around this age. Mice were caged individually in transparent polyethylene cages (35 cm × 12 cm × 12 cm), which were equipped with pine chip bedding and a polyvinyl chloride housing tube (5 cm diameter, 8 cm length). Food pellets (Purina Rodent Chow, no. 5001) were weighed prior to placement in each cage. Tap water was available ad libitum. Mice were housed under a 12/12 hour reversed light/dark cycle with lights off from 09:30 -21:30. All testing was completed during the dark phase. All test procedures were approved by the Dalhousie Committee on Animal Care. Mice were genotyped for the APP and PS1 transgenes using tissue samples from ear-punches, using PCR by Chris Sinal (Department of Pharmacology, Dalhousie University; Chapter 2).

3.3.2 Collection of blood and glucose at 12 months of age

Mice were fasted for a minimum of 8 hours and deeply anesthetized with sodium phenobarbital through intraperitoneal injections. After complete anesthetization, blood was collected via cardiac puncture using 21 or 25 gauge needles, depending on mouse size. One drop of blood was placed on a glucose reader (Accu-chek) in order to read glucose concentration in blood plasma. Blood was then placed in prepared tubes that contained a solution of 10% EDTA and aprotenin® which were kept in an ice bucket until they were centrifuged at 1,000g for 10 minutes at 4 °C. Supernatant (plasma) was transferred into a new tube, 10% HCl was added to prevent degradation, and plasma samples were stored at -80 °C. Using t-tests, we compared glucose concentrations between genotypes. We considered significance if $p<0.05$.

3.3.3 Fat and muscle mass at 12 months of age

Perigonadal and visceral white adipose tissue was dissected and weighed (Figure 20). Interscapular brown adipose tissue (BAT) was also dissected and weighed. The interscapular brown adipose tissue was selected as it contains the highest quantity of brown fat in mice (Smith et al., 2013). The upper and lower portion of the gastrocnemius

muscle was dissected and weighed with an electronic balancer (Ohaus, Maryland; Figure 21). The mean weights of WAT, BAT, and muscle tissue were calculated and compared for genotype differences. T-tests used to compare WAT, BAT, and lean muscle mass between genotypes. We considered significance if $p<0.05$.

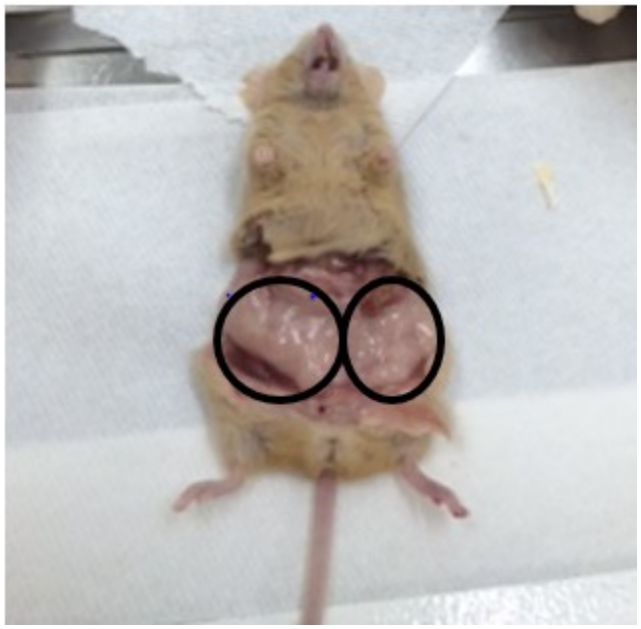

Figure 20 Fat tissue was extracted from abdominal cavity. Perigonadal fat was dissected.

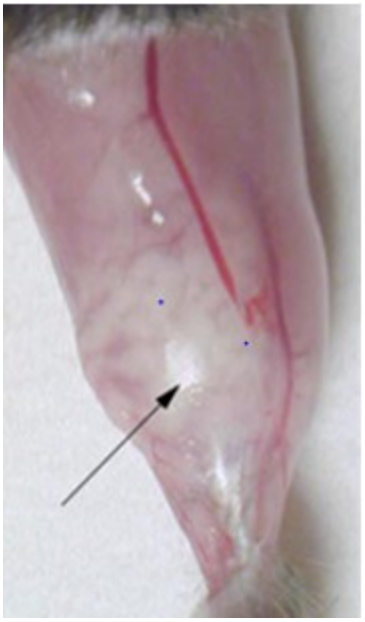

Figure 21 This is the gastrocnemius muscle that was taken from both mouse genotypes. (From Lefesvre et al., 2002).

3.3.4 Insulin concentration

Plasma insulin was measured using ELISA kits purchased from Millipore (St. Charles, Missouri, catalogue number: EZML-82K). Kits were supplied with 96-welled plates pre-coated with mouse monoclonal anti-rat insulin antibodies, 10X HRP wash buffer, standards, matrix solutions, assay buffer, insulin detection antibody, enzyme solution, stop solution, and substrate. The lowest amount of insulin that can be detected in the kit used is 0.2 ng/mL when the sample size is 10 μL. Specificity for mouse/rat insulin in this kit is 100%.

A 10X washer buffer was diluted with 450 mL of deionized water to make a diluted wash buffer. This diluted washing buffer was used to wash the ELISA plate (300 μL/well). An assay buffer (10 μL) was added to all non-specific binding and sample wells. We then added 10 μL of matrix solution to non-specific binding and standard wells. Serially diluted insulin standards were placed in duplicates (10 μL/well). We then placed 10 μL of sample in the remaining wells, not designated as non-specific binding and standard wells, in duplicates. A detection antibody reagent (80 μL) was added to all wells. Afterwards, the plate was sealed and incubated at room temperature. The plate was then shaken for 2 hours at 500 rpm. Afterwards, contents in plate were decanted and plate was washed 3 times with diluted washing buffer (300 μL/well). Approximately 100 μL of enzyme solution was added to each well. The plate was then sealed, incubated at room temperature, and shaken for 30 minutes. The plate was decanted again and washed 6 times with diluted washing buffer (300 μL/well). We then added substrate solution to each well (100 μL). We then sealed the plate and shook it for 15 minutes, after which 100 μL of stop solution was added. The Varioskan™ flash multimode reader was used to measure fluorescence emitted from the plate. We set the wavelength of the Varioskan™ flash multimode reader at 450 nm. Data were analyzed using the graphpad prism program. We compared insulin concentration between 5xFAD mice and WT mice using a t-test. We considered significance if $p<0.05$.

3.3.5 Quantitative PCR measures of leptin, HSL, UCP-2, and UCP-1

White and brown adipose tissue (WAT and BAT) were collected from 6 WT and 6 5xFAD (5 for WAT) female mice at 12 months of age. WAT tissue was analyzed for leptin, HSL, and UCP-2 mRNA, and BAT tissue was analyzed for UCP-1 mRNA

expression via qPCR analysis (primer sequences are provided in Table 2). We extracted RNA from WAT and BAT via PureZOL™ RNA isolation reagent as well as the Aurum™ Total RNA Fatty and Fibrous Tissue Kit (Bio-Rad, Hercules, CA, catalogue number: 732-6830). We reverse transcribed the extracted RNA via the iScript™ cDNA Synthesis Kit (Bio-Rad, Hercules, CA, catalogue number: 170-8890). Final products were analyzed using the quantitative real-time PCR using iQ™ SYBR (Green Supermix (Bio-Rad, Hercules, CA, catalogue number: 170-8880). Primers were purchased from Integrated DNA Technologies (Skokie, IL). The reference primer used was 18S. Enzyme activation occurred at 98 °C for 30 seconds and denaturation occurred at 98 °C for 5 seconds. Annealing and extension of cDNA occurred at 60, and 72 °C for 20 and 30 seconds. We also annealed cDNA at 72 °C for 5 minutes. The melt curve was set from 65 to 95 °C where temperature rose 0.5 degrees every 5 seconds. Forty cycles were done during annealing. We normalized our qPCR data to our reference gene, ribosomal 18S. Under the umbrella of relative quantification, we used the $\Delta\Delta C_T$ method (Haimes and Kelly, 2014). To calculate the $\Delta\Delta C_T$ we took the difference between mean C_T (the threshold cycle is the time of cycle when DNA amplification is observable) scores between our reference gene and target gene within either the WT or 5xFAD mice, and then the difference between the ΔC_T of the genotypes was calculated, giving us the $\Delta\Delta C_T$. We inserted the $\Delta\Delta C_T$ in $2^{-\Delta\Delta C_T}$ to give us the expression fold. A t-test was done comparing the ΔC_T between genotypes. For the interests of simplicity, the more negative the ΔC_T, the less expression, the more positive the ΔC_T means elevated expression. We considered significance if $p<0.05$.

3.3.6 Correlational analysis

A correlational analysis (Pearson's r) was used to evaluate the strength of relationship between white adipose tissue, food intake, and body weight. White adipose tissue was compared to food intake in all food presentations. This was done as we collected white adipose at 12 months of age. At that time, mice were presented with food on hopper, food in cage, and mashed (see Chapter 2). Hence, the quantity of white adipose tissue would have been affected by all these food presentations. Consequently, we compared white adipose tissue mass to food intake and body weight in all food presentations.

3.4 RESULTS

3.4.1 5xFAD mice show less white adipose tissue but no difference in brown adipose tissue or lean muscle mass compared to WT mice

The 5xFAD mice (n= 21) had significantly less WAT compared to WT (n=26) controls ($t = 4.125$, $df = 46$, $p<0.0003$; Figure 22A). There were no significant differences in brown adipose tissue weight between genotypes ($t= 0.2653$, $df= 46$ $p>0.05$; Figure 22B). No differences between genotypes were seen in weight for the upper ($t=0.07215$, $df = 46$, $p>0.05$) or lower ($t = 0.6119$, $df= 46$, $p>0.05$) portion of the gastrocnemius muscle (Figure 23A and 23B).

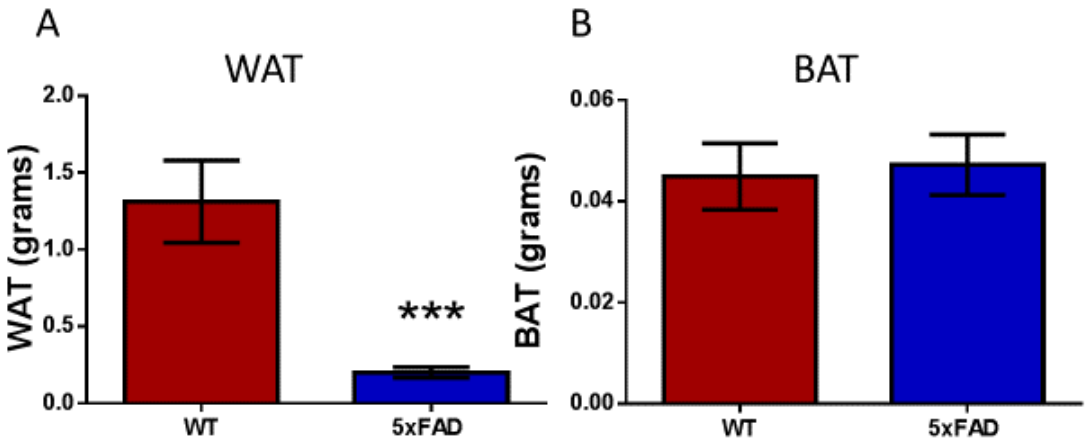

Figure 22 WT (n=26) mice had more WAT than 5xFAD (n=21) mice (p<0.001). No difference in BAT mass was seen between genotypes (p>0.05).

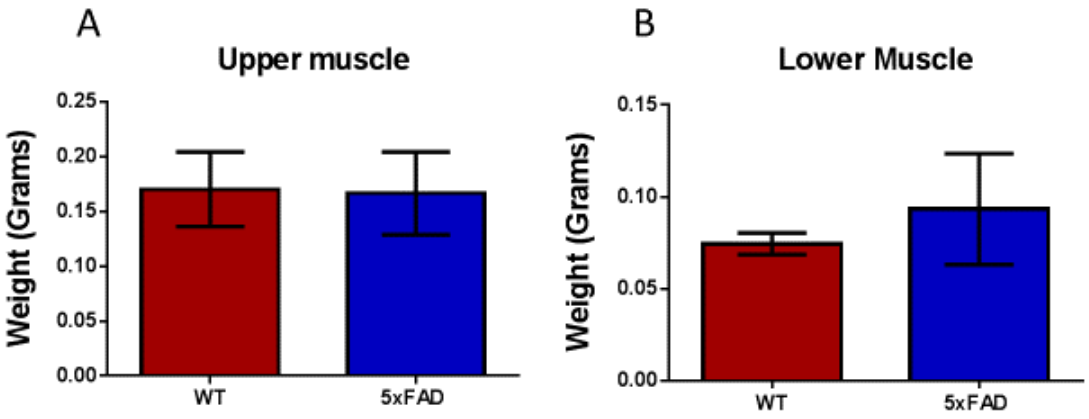

Figure 23 Mean weights (g) of the upper (A) and lower (B) portions of the gastrocnemius muscle showed no significant differences between WT (n= 26) and 5xFAD (n= 21) mice.

3.4.2 Insulin and glucose concentrations did not differ between genotypes

Insulin concentration did not differ between fasted genotypes (t =0.3145, df= 46, p>0.05; Figure 24A) nor did levels of glucose differ between fasted genotypes (t = 1.735, df= 46, p>0.05; Figure 24B). The glucose concentration measured for each genotype fell within the normal range of fasting glucose for each strain (6.2 mmol/L for C57BL/6JxSJL/J F1 and 5.55 mmol/L for 5xFAD mice; Goren et al., 2004; Kim et al., 2013).

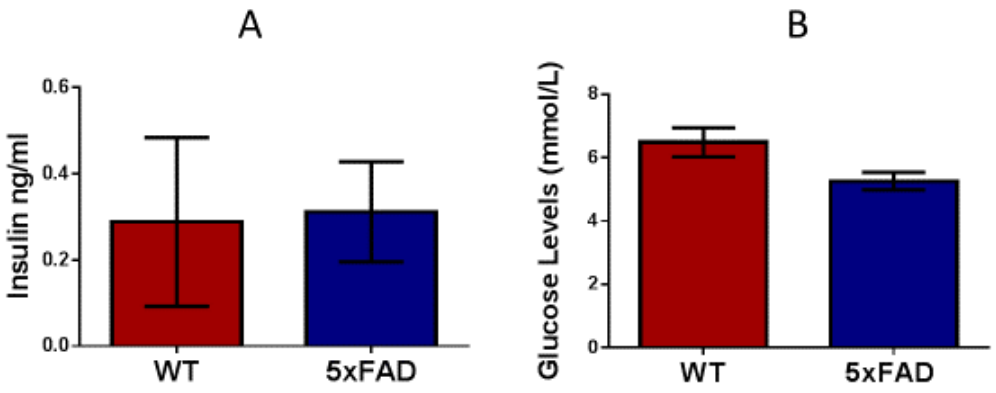

Figure 24 No significant differences were seen between WT (n= 26) and 5xFAD (n=21) mice in concentrations of insulin or glucose (p>0.05).

3.4.3 qPCR analysis showed that 5xFAD mice had reduced expression of mRNA for leptin, HSL, and UCP-2, but increased expression of UCP-1

The qPCR analyses found 5xFAD mice exhibited a 62 fold decrease (refer to method section 3.3.5) in expression of leptin mRNA in WAT (t=2.74, df = 11, p=0.038; Figure 25A). The $\Delta\Delta C_T$ for leptin expression was 5.96 +/- 2.2.

Our transgenic mice showed a 49.7 fold decrease in expression for HSL in WAT (t =3.34, df = 11 p=0.0248; Figure 25B). The $\Delta\Delta C_T$ for HSL expression was 5.63 +/- 1.7.

UCP-2 expression in WAT was reduced 62 fold in the 5xFAD mice (t=3.807, df =5.313, p= 0.012, $\Delta\Delta C_T$= 5.96 +/- 1.6; Figure 25C).

UCP-1 expression in BAT was reduced by 3 fold in 5xFAD mice (t= 2.55, df=6.878, p=0.0386, $\Delta\Delta C_T$= -1.63 +/- 0.64; Figure 26).

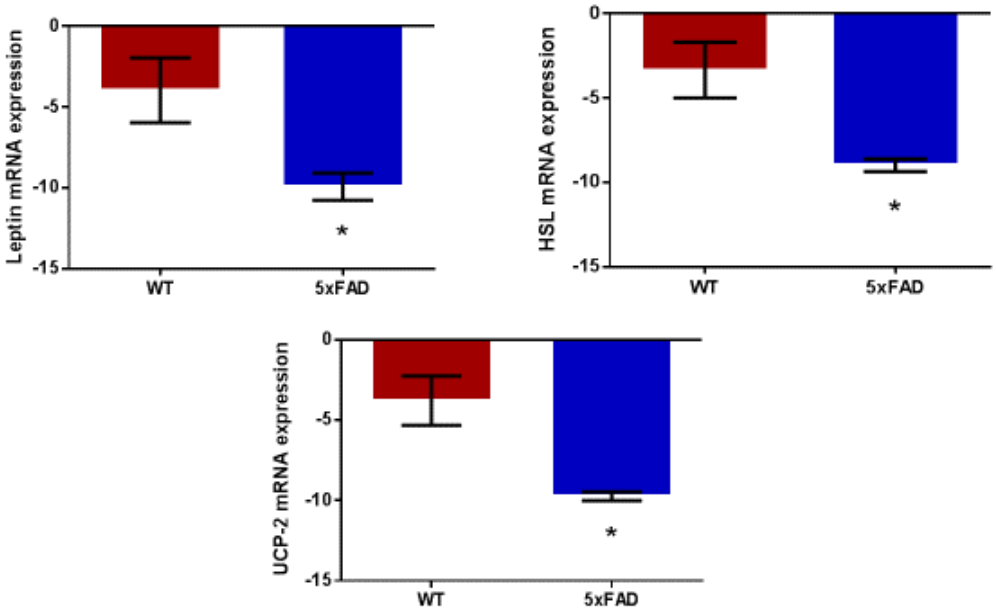

Figure 25 mRNA expression of leptin, HSL, and UCP-2 in WAT. The above plots depict ΔC_T scores. All plots show that the 5xFAD mice have less expression of leptin, HSL, and UCP-2 than WT mice ($p<0.05$). Recall that the more negative ΔC_T, the less expression, and the more positive the ΔC_T leads to elevated expression

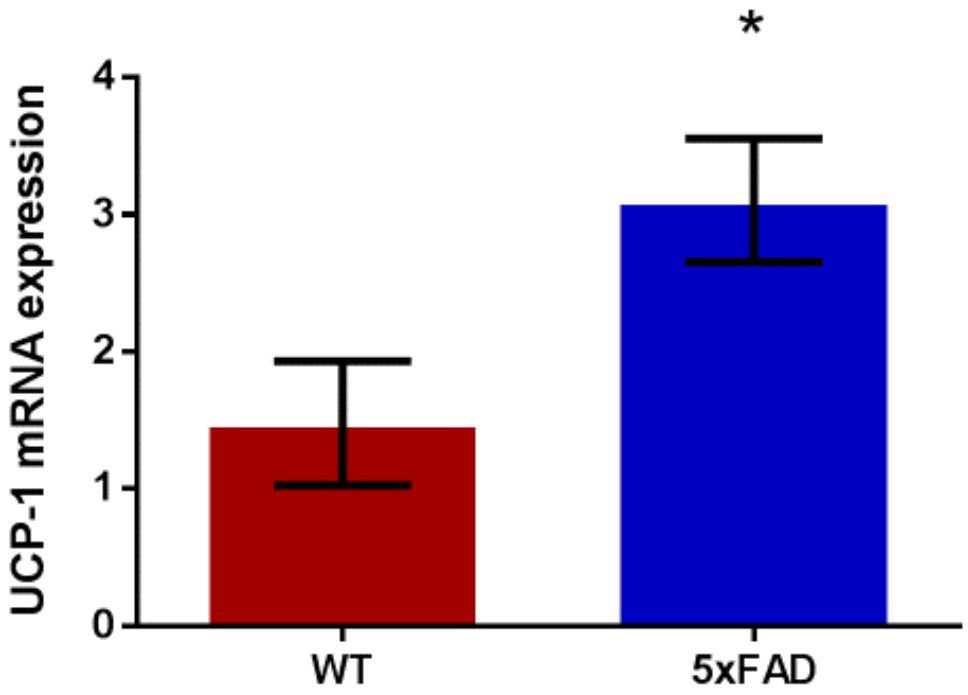

Figure 26 mRNA expression of UCP-1 in BAT. The above plot depicts ΔC_T scores. Note that 5xFAD mice express more UCP-1 than WT mice ($p<0.05$). Recall that the more negative ΔC_T, the less expression, and more positive ΔC_T indicates elevated expression.

3.4.4. Correlational analyses showed no significant relationship between WAT and body weight for either genotype and a significant correlation between WAT and food intake for WT but not for 5xFAD mice

A correlation was done to evaluate the relationship between WAT, food intake, and body weight. There was no significant correlation between WAT and food intake in WT mice (n=26, r = 0.246, *df* = 63, *p* = 0.34, R^2= 0.061) or in 5xFAD mice (n= 21, r = 0.152, *df* = 63, *p* = 0.637 R^2= 0.023). WAT and body weight showed a significant correlation for the WT mice (r = 0.953, *df* = 63 *p* = 0.001, R^2 = 0.91) but not for the 5xFAD mice (r = 0.372, *df* = 63 *p* = 0.233, R^2= 0.14; Figures 27A and 27B).

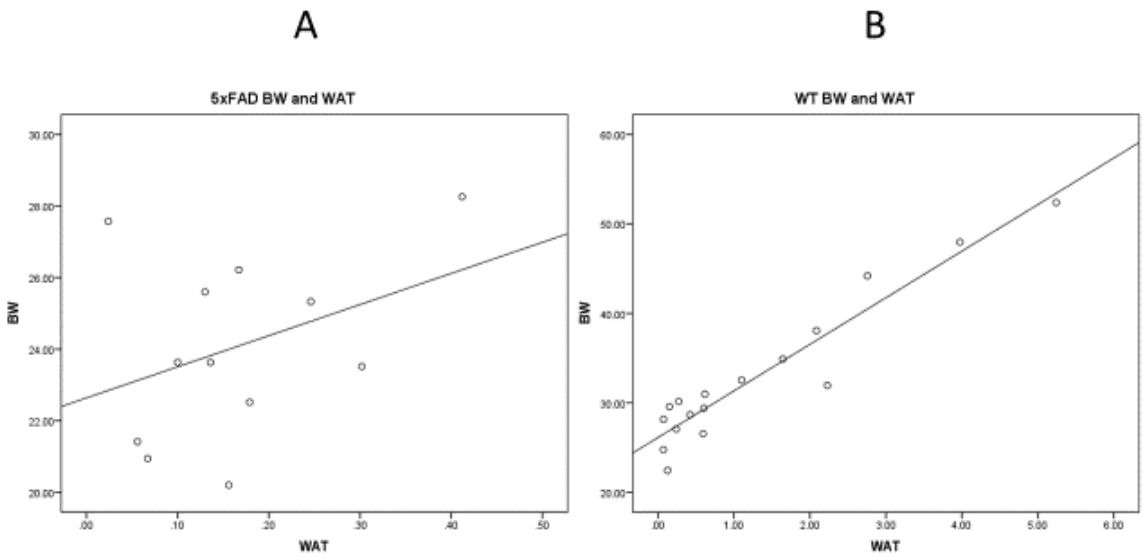

Figure 27 Correlation of body weight (BW) and white adipose tissue (WAT) mass in 5xFAD and WT mice. WAT and body weight did not show a significant correlation in 5xFAD mice ($r = 0.372$, $df = 63$ $p = 0.233$, $R^2 = 0.14$; A). WAT and body weight showed a significant correlation for the WT mice ($r = 0.953$, $df = 63$ $p = 0.001$, $R^2 = 0.91$; B).

3.5 DISCUSSION

3.5.1 WAT reduction in 5xFAD mice but no loss of muscle mass

From the above results, there is a significant reduction in white adipose tissue in the 5xFAD mice ($p<0.05$). This reduction in fat mass also occurs in AD patients (Renvall et al., 1993), indicating that the 5xFAD mouse reflects this particular phenotype of weight loss seen in AD. No differences were seen in BAT mass between genotypes ($p>0.05$). This is not surprising as there are no clinical reports supporting abnormalities in brown fat mass in AD patients or mouse models of AD. There were no differences in the gastrocnemius muscle weight between genotypes. This conflicts with symptoms seen in AD patients and some mouse models of AD such as Tg2576 mice which found reduced lean muscle mass (Ishii et al., 2014; Lowry, 2010). Our measurements of muscle mass were crude, however, as they did not account for lean muscle mass throughout the body. Consequently, measuring the gastrocnemius muscle only provided a narrow view of muscle content in these mice. It is important for future purposes to use a DEXA scanner (PIXImus2; Lunar, Madison, WI). This will provide a holistic view of body composition in the 5xFAD mouse model.

3.5.2 Insulin and glucose levels did not differ between genotypes

We found no differences in insulin or glucose concentrations between genotypes and glucose concentrations were within normal range for each mouse strain (Goren et al., 2004; Kim et al., 2013). Because insulin regulates plasma glucose levels, these results indicate that insulin resistance is unlikely in the 5xFAD mice, as we would see abnormalities in glucose levels if there was insulin resistance. These findings do not support the observations of Craft et al. (1998), who found elevated insulin levels in the plasma of AD patients. However, there may be insulin resistance in the brain of the 5xFAD mice and our study did not investigate this. The 5xFAD mouse model exhibits neuroinflammation (Bilkei-Gorzo, 2014) and shows elevated levels of TNF-α, a cytokine that is involved in the phosphorylation of serine residues of insulin receptors (Ano et al., 2015; Bomfim et al., 2012). TNF-α inhibits the activation of insulin receptors, impairing normal insulin signaling (Bomfim et al., 2012). Hence, insulin resistance in the brains of 5xFAD mice may be present, and should be investigated.

3.5.3 Leptin, UCP-2, and HSL reduction in WAT of 5xFAD mice

Our results showed that the 5xFD mice exhibited reduced expression of leptin, UCP-2, and HSL in their WAT. These results would be expected as leptin is highly correlated with fat mass (Sørensen et al., 1996) and the 5xFAD mice have significantly reduced fat mass. This phenotype is also seen AD patients who exhibit reduced levels of leptin and fat mass (Lieb et al., 2009). It is not surprising that UCP-2 expression is reduced as leptin is responsible for the up-regulation of UCP-2 (Zhang et al., 2008), and reduced leptin leads to reduced expression of UCP-2. The reduced expression of HSL is also not surprising as this hormone regulates PPARγ signaling (peroxisome proliferator-activated receptor; Zechner et al., 2014). PPARγ is a receptor that regulates adipogenesis or lipid synthesis (Zechner et al., 2014). HSL-deficient mice exhibit lipodystrophy and weight loss. This is because substrates such as fatty acids and glycerol that are produced by HSL and activate PPARγ, are reduced due to the hypoactivity of HSL (Zechner et al., 2014).

3.5.4 Elevated UCP-1 expression occurs in BAT from 5xFAD mice

UCP-1 was significantly elevated in 5xFAD mice. This protein regulates thermogenesis and fat burn (Yo et al., 2013). This occurs because UCP-1 separates oxidative phosphorylation from ATP synthesis leading to the production of heat (Arsenijevic et al, 2000). Both humans and mice that have elevated metabolism show higher BAT and UCP-1 activation (Yo et al., 2013). Indeed, elevated activation of BAT and UCP-1 prevents obesity (Sharma et al., 2014; Kozak et al., 2010). Hence, elevated expression of UCP-1 in the 5xFAD mouse model likely contributes to the selective and pronounced loss of WAT in this mouse model.

3.5.5 General discussion

There is a significant decline in white adipose tissue and markers for adipogenesis in the 5xFAD mice, indicating that adipogensis is hindered in this mouse model of AD. UCP-1 expression is elevated in this model, indicating that fat burn is increased in the 5xFAD mouse model. Thus, it appears that both reduced adipogenesis and elevated fat burn contribute to WAT loss and to reduced body weight in the 5xFAD mouse model.

A limitation to this study is the crude measurements of body composition. A DEXA scanner (PIXImus2; Lunar, Madison, WI) would allow us to evaluate all WAT and lean muscle mass in mice, giving us an accurate picture of body composition in the 5xFAD mice. Measuring the gastrocnemius muscle may not give us a holistic picture of lean muscle mass in this mouse model. Indeed, both AD patients and other mouse models, such as the Tg2576, exhibit reduced lean muscle mass (Ishii et al., 2014; Lowry, 2010).

Future directions of this study include using metabolic calorimetry cages to accurately measure energy expenditure. Metabolic cages will provide information about the activity level, volume of oxygen consumed, body temperature and food consumed. This would provide a metabolic characterization of 5xFAD mice especially if the weight loss observed is due to decreased caloric intake or increased energy expenditure. To accurately estimate fat mass, we intend to measure body composition using a DEXA scanner (PIXImus2; Lunar, Madison, WI). This system will provide quantitative data on the fat tissue content, the lean tissue content, and the total tissue mass within the region of interest (ROI). An experiment of this caliber will provide detailed characterization of fat distribution and lean tissue in AD mice. Based on the results above and from chapter 2, we believe a possible explanation for the reduced body weight in 5xFAD mice may be the dysfunction of the gut-brain axis regulating energy homeostasis. Consequently, future studies must also investigate plaque load and functionality of neurons that regulate thermogenesis, feeding, and body weight. For instance, BAT activity is controlled by sympathetic circuits originating from the central nervous system (Tupone et al., 2014). Specifically, cutaneous thermal sensory receptors activate or inhibit medial and median preoptic area neurons which themselves mediate the activation or inhibition of dorsal and dorsomedial hypothalamic neurons (Tupone et al., 2014). These hypothalamic neurons signal BAT sympathetic preganglionic neurons to innervate BAT sympathetic postganglionic neurons (Tupone et al., 2014). Norepinephrine secreted from the postganglionic neurons binds to adrenergic receptors that activate BAT (Tupone et al., 2014). Consequently, analyzing plaque load and c-Fos expression in the preoptic area, dorsomedial and dorsal hypothalamic areas will give us insight into whether thermogenesis signaling is impaired, explaining for elevated UCP-1 expression.

We also wish to measure c-Fos expression and plaque load in the arcuate nucleus of the hypothalamus (a region of the brain that regulates energy expenditure, food intake, and body weight) to see if neurons in 5xFAD mice are activated by hormones that regulate food intake and body weight (i.e. leptin and insulin; Barsh and Shwartz, 2002). The arcuate nucleus of the hypothalamus regulates information from hormones that mediate food intake and body weight, such as leptin and insulin. Due to the fact that we see feeding and body weight abnormalities in the 5xFAD mouse model, it is important to investigate the functionality of populations of neurons that regulate food intake and body weight such as POMC, NYP, and CART neurons (all located in the arcuate nucleus of the hypothalamus; Barsh and Shwartz, 2002). These tests may provide insight as to whether hormone pathways that regulate hunger and food intake are hypoactive in the 5xFAD mice, or if hormone pathways that regulate satiation and feeding inhibition are hyperactive in the 5xFAD mice.

3.5.6 Conclusion

The 5xFAD mouse model of AD shows significant reductions in WAT but not BAT or lean muscle mass. This mouse model exhibits reductions in the expression of leptin, UCP-2, and HSL. This supports the conclusion that adipogenesis is likely reduced in the 5xFAD mouse model. High levels of UCP-1 are expressed in the 5xFAD mouse model, suggesting that these mice undergo significant fat burn or thermogenesis. Consequently, these mice appear to have reduced adipogenesis and elevated thermogenesis resulting in reduced WAT and low body weight.

3.6 Acknowledgments

This project would be unable to happen without the assistance and funding of NSERC. The assistance and guidance from Drs. Richard E. Brown and Younes Anini have been nothing short of brilliant. Thanks to Stephanie Pelletier and Michael Landsman for assisting in running the experiments and providing feedback for experimental design. Drs. Aimée Wong and Kurt Stover were exceptional in providing sound feedback on statistical tests and experimental design.

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3.8 Supplementary Table

Table 2 Forward and reverse primers used for IQ SYBR green qPCR assay. The listed primers are targets for specific target genes, such as leptin, UCP-1, UCP-2, and HSL. Primers were also generated to target ribosomal 18S, a reference gene expressed throughout the body. Primers are important for the annealing process during amplification of cDNA.

Target Gene	Forward Primer	Reverse Primer
Leptin	TGCTGAAGTTTCAAAGGCCACCAG	ATGCCTTTGGATGGGTGGTCTACA
UCP-1	TGGAATAGCGGCGTGCTTG	CTCATCAGATTGGGAGTAG
UCP-2	AGCCTACAAGACCATTGCACGAGA	AGGTCATCTGTCATGAGGTTGGCT
HSL	TGTGGCACAGACCTCTAAATCCCA	TCTCCAGGTGAACCAAGCAGTTCA
18S	TCAACTTTCGATGGTAGTCGCCGT	TCCTTGGATGTGGTAGCCGTTTCT

CHAPTER 4 DISCUSSION

4.1 5xFAD MICE DIFFER FROM WT MICE IN FOOD INTAKE, BODY WEIGHT, ACTIVITY, FRAILTY, TISSUE COMPOSITION, AND EXPRESSION OF MARKERS FOR ADIPOGENESIS AND THERMOGENESIS

The data in chapters 2 and 3 highlight the significant differences in body weight, food intake, and metabolic hormones between the 5xFAD and their WT counterparts at 12 months of age. Compared to WT controls, 5xFAD mice exhibit overall reduced food intake, body weight, and activity levels, as well as reduced WAT, and reduced expression for leptin, UCP-2, and HSL. The 5xFAD mice score higher on frailty and show elevated expression for UCP-1, an important marker and regulator for thermogenesis (Vidal-Puig et al., 1997). AD patients exhibit reduced food consumption and body weight as seen in the 5xFAD mouse model (Sergi et al., 2013). The 5xFAD mice showed reduced climbing and rearing. This not surprising considering that at 9 months of age motor coordination issues arise in the 5xFAD mouse model (O'Leary, 2013). AD patients also have reduced motor skills as they age (Buchman and Bennett, 2011). The 5xFAD mouse model also displayed frequent and lengthy inactivity behaviour compared to their WT counterparts. This may be reflective of catatonic behaviour or lack of activity seen in AD patients (Jerry, 2006). Increased frailty scores are seen in AD patients, as seen in their motor skill, weight, and appetite loss (Buchman and Bennett, 2011; Koch et al., 2013). The 5xFAD mice also showed a significant increase in frailty score compared to their WT counterparts. Food presentations did not alter frailty scores showing that food accessibility does not influence frailty. Different types of food presentation also showed no effect on food intake or body weight overall.

There was reduced WAT in the 5xFAD mice compared the WT mice, although no differences were seen in gastrocnemius muscle weight. Loss of WAT is mirrored in AD patients as they show signs of reduced fat mass (Renvall et al., 1993). Reduced expression of leptin, UCP-2, and HSL in the 5xFAD mice may contribute to loss of WAT and impairments in food intake in this mouse model. Indeed, leptin is a hormone that regulates body weight and food satiation by activating POMC and CART neurons and inhibiting NPY and AgRP neurons in the arcuate nucleus of the hypothalamus leading to

decreased food intake and increased energy expenditure (Barsh and Schwartz, 2002; Coll and Yeo, 2013; Zeltzer et al., 2012). Leptin levels are positively correlated with fat mass, in that more WAT or adipocytes produce more leptin, making leptin a marker for adiposity (Sořrensen et al., 1996). Leptin has neurotrophic effects in that it promotes cell survival (Guo et al., 2008). Reduced leptin, as seen in AD patients, may increase neuronal susceptibility to apoptosis (Lieb et al., 2009). However, studies using Tg2576 mouse strains, show that there is no change in expression of NPY and AgRP under low leptin levels (Ishii et al., 2014). This is problematic as low leptin levels should elevate expression of these proteins (Zeltzer et al., 2012). However, NPY and AgRP neurons are influenced by multiple hormones (i.e. insulin and ghrelin), thus compensation for their activation or deactivation may occur without leptin (Zeltzer et al., 2012). Consequently, it may be a contribution of low leptin levels and unchanged expression of NPY and AgRP that leads to food intake abnormalities, weight loss, and exacerbated cell death in the brain (Coll and Yeo, 2013; Zeltzer et al., 2012).

UCP-2 is a mitochondrial protein found in the inner mitochondrial membrane and is predominantly expressed in white adipose tissue (Vidal-Puig et al., 1997). Its function is similar to UCP-1 in that it separates oxidative phosphorylation from adenosine triphosphate synthesis, resulting in the production of heat. Consequently, UCP-2 regulates thermogenesis (Arsenijevic et al, 2000). Furthermore, UCP-2 limits the production of reactive oxidative species and knockout mice lacking UCP-2 display elevated concentrations of reactive oxidative species (Arsenijevic et al, 2000). UCP-2 is up-regulated by leptin (Zhang et al., 2008) and if leptin levels are reduced, then expression of UCP-2 should also be reduced. Our data showed that the 5xFAD mice had low levels of leptin and therefore expressed little UCP-2. Like leptin, UCP-2 is a marker for adiposity.

Hormone sensitive lipase (HSL), is a marker for adipose catabolism found in white and brown adipose tissue (Zechner et al., 2014). HSL regulates free fatty acid secretion from adipose tissue into the circulation (Haemmerle et al., 2001). Consequently, constant activation of this protein leads to leaner body types (Cummings et al., 1996). On the other hand, HSL deficient mice exhibit lipodystrophy, reduced body weight, and reduced adipogenesis, because HSL regulates PPARγ signalling. PPARγ is a receptor that

regulates adipogenesis or lipid synthesis. HSL produces important substrates (i.e. fatty acids) that activate PPARγ (Zechner et al., 2014). Without these substrates, PPARγ activation is reduced and so is adipogenesis.

UCP-1 is a mitochondrial protein found in the inner mitochondrial membrane. This protein is predominately found in brown adipose tissue (BAT; Yo et al., 2013). Elevated expression of UCP-1 has been associated with thermogenesis (converting ATP to heat in order to prevent hypothermia) and fat burn (Yo et al., 2013). Furthermore, humans and mice that have elevated metabolism exhibit elevated BAT and UCP-1 activation and expression (Yo et al., 2013). Activation of BAT and UCP-1 is regulated by neurons within the medial preoptic area and dorsal medial hypothalamus (Tupone et al., 2014) However, AD patients show no elevated expression of UCP-1 (Cornelius et al., 2013). We have shown that the 5xFAD mice display elevated expression for UCP-1, indicating elevated thermogenesis and fat burn. Elevated expression for UCP-1 may be a result of hyperactivated neurons within the medial preoptic area and dorsal medial hypothalamus.

Although we found no significant differences in plasma insulin levels (as seen in AD patients; Craft et al., 1998) in the 5xFAD mice compared to the WT mice, there may still be insulin resistance present in the brain. Indeed, the 5xFAD mouse model exhibits neuroinflammation (Bilkei-Gorzo, 2014). Consequently, they show elevated levels of TNF-α, a cytokine that is involved in the phosphorylation of serine residues of insulin receptors (Ano et al., 2015; Bomfim et al., 2012). This consequently inhibits the activation of these insulin receptors, impairing normal insulin signaling (Bomfim et al., 2012). Hence, insulin resistance in the brains of 5xFAD may be present, however this must be investigated.

4.2 Age differences seen between 3 and 12 months of age with regard to food intake and body weight in the 5xFAD mice

At 3 and 6 months of age there were no differences in body weight or feeding between 5xFAD mice and WT mice. However, a significant reduction in food intake and body weight occurred at 9 to 12 months of age in the 5xFAD mice compared to WT mice. This reduction in food intake and body weight in the 5xFAD mice occurs during

their middle age (270-365 days; Rae and Brown 2015) or in human years between 38-47 years old (Lifespan as a biomarker, 2011). Most patients with familial early onset Alzheimer's disease do not develop symptoms until approximately 65 years of age (Zigmond et al., 2015). However, the 5xFAD mouse is specifically designed to produce excessive plaques. This consequently allows for symptoms to occur or surface at an earlier age. No differences between genotypes were seen at 3 or 6 months of age. This is not surprising considering mice are adults at this stage and unlikely to exhibit AD like symptoms (Lifespan as a biomarker, 2011). Consequently, as mice age, phenotypic differences begin to be seen, just as occurs in patients suffering from AD, at the later stage of life (Lieb et al., 2009). Different methods of food presentation did not alter weight loss, showing that accessibility to food resources does not influence food intake or body weight in the 5xFAD mouse model of AD. This reduction in food consumption and reduced weight can be explained by the amyloid cascade hypothesis or the neuroinflammatory hypothesis. The amyloid cascade hypothesis suggests that amyloid plaques oligeromize and inhibit processes that would normally instruct cells to survive (De Felice et al., 2009). Disrupting pathways such as PI3K generally leads to cell death (De Felice et al., 2009). Consequently, if plaques are found in hypothalamic regions that regulate food intake and body weight, it may explain why these mice lose appetite and weight. The inflammatory hypothesis suggests that the AD brain undergoes a neuroinflammatory state where pro-inflammatory factors such as TNF-α inhibit the PI3K pathway and promote cell death (Bomfim et al., 2012). The 5xFAD mice show neuroinflammation and elevated TNF-α (Bilkei-Gorzo, 2014; Bomfim et al., 2012). However, neuroinflammation only occurs in areas that have plaques, as plaques attract microglia (Mohandas et al., 2009). Thus, if there are signs of neuroinflammation in hypothalamic regions that regulate food intake and body weight, this may explain reduced food consumption and body weight seen in the 5xFAD mouse model.

4.3 THE 5XFAD MICE ARE GOOD MODELS FOR THE PERIPHERAL AD-LIKE SYMPTOMS BASED ON FACE AND CONSTRUCT VALIDITY

The 5xFAD mice show promise as a model for the peripheral symptoms seen in AD patients. Loss of appetite and body weight seen in AD patients is also seen in 5xFAD

mice (Barrett-Connor et al., 1996; Sergi et al., 2013). Furthermore, reduced leptin expression in the 5xFAD mice is seen in AD patients (Lieb et al., 2009). WAT loss seen in the 5xFAD mice is also seen in AD patients who exhibit significant reductions in fat mass (Renvall et al., 1993). Like AD patients, the 5xFAD mice show a significant increase in frailty scores, catatonic behaviour, and motor deficits (Alzheimer's Caregiver Resources.com; Buchman and Bennett, 2011; Koch et al., 2013). Although these mice show no abnormalities of plasma insulin, as seen in AD patients, they still may have insulin resistance in their brain due to neuroinflammation (Bilkei-Gorzo, 2014; Craft et al., 1998). Lack of reduction in lean muscle in 5xFAD mice may be a limitation of the study as we only measured the gastrocnemius muscle in the 5xFAD mice. Patients with AD show loss of lean muscle (Lowry, 2010). Hence, this mouse model may exhibit lean muscle loss in other lean muscle tissues rather than the gastrocnemius. A significant difference between AD patients and the 5xFAD mice is that 5xFAD mice have increased expression of UCP-1 whereas AD patients show no increased expression for this protein (Cornelius et al., 2013). However, Cornelius et al. (2013) do not specify the type of AD their patients suffer from. This means that it is possible that patients with familial early onset AD may exhibit elevated UCP-1 expression. Overall we believe that the 5xFAD mouse model of AD provides reflective face and construct validity for symptoms seen in patients in AD.

4.4 Future directions and limitations

During our initial investigation, we found that the 5xFAD mouse model exhibits progressive weight loss and significant reduction of WAT compared to WT littermates. Decreased body weight and fat mass can be due to reduction in food intake or increased energy expenditure. Our results indicated that 5xFAD mice showed a reduction in their food intake and reduced energy expenditure. To further confirm these results, we will use calorimetry cages to measure food intake, activity, and energy expenditure more accurately. Metabolic cages will provide information about the activity level, volume of oxygen consumed, body temperature, and food consumed. The results of the proposed PhD experiments will provide a metabolic characterization of 5xFAD mice especially if

the weight loss observed is due to decreased caloric intake or increased energy expenditure.

In order to accurately estimate fat and lean muscle mass, we intend to measure body composition using a DEXA scanner (PIXImus2; Lunar, Madison, WI). This system will provide quantitative data on the fat tissue content, the lean tissue content, and the total tissue mass within the region of interest (ROI). An experiment of this caliber will provide detailed characterization of fat distribution and lean tissue in 5xFAD mice. Based on the results from chapter 2 and 3, we believe a possible explanation for the reduction of body weight in the 5xFAD mice may be due to impairment in the gut-brain axis regulating energy homeostasis. Consequently, this study must also investigate plaque load and functionality of neurons that regulate thermogenesis, feeding, and body weight. For instance, BAT activity is controlled by sympathetic circuits originating from the central nervous system (Tupone et al., 2014). Specifically, cutaneous thermal sensory receptors activate or inhibit medial and median preoptic area neurons which themselves mediate the activation or inhibition of dorsal and dorsomedial hypothalamic neurons (Tupone et al., 2014). These hypothalamic neurons regulate BAT activation (Tupone et al., 2014). Consequently, analyzing plaque load and c-Fos expression in the preoptic area, dorsomedial and dorsal hypothalamic areas will give us insight as to whether thermogenesis signaling is impaired, explaining for elevated UCP-1 expression.

We will also measure c-Fos expression and plaque load in the arcuate nucleus of the hypothalamus (a region of the brain that regulates information from hormones, such as leptin and insulin, that mediate food intake, body weight, and energy expenditure) to see if neurons are still activated by hormones that regulate food intake and body weight (Barsh and Shwartz, 2002). Due to the fact that we see feeding and body weight abnormalities in the 5xFAD mouse model, it is important to investigate the functionality of populations of neurons that regulate food intake and body weight such as POMC, NYP, and CART neurons (all located in the arcuate nucleus of the hypothalamus; Barsh and Shwartz, 2002). These sets of experiments on the 5xFAD mouse model will either support or contradict the theory that AD is a cognitive metabolic syndrome. This theory suggests that the brain of AD patients exhibit reduced cerebral glucose metabolism and insulin resistance (Erol, 2010; Nordberg, 2014). In the periphery, insulin levels are

abnormally elevated and leptin levels are reduced (Erol, 2010; Lieb et al., 2009). These abnormalities lead to cell death. Cell death is a consequence of reduced activation of the PI3K pathway (a pathway responsible for cell survival) from insulin and leptin (Erol, 2010; Lieb et al., 2009). Elevated insulin in the periphery leads to over activation of the Ras/MAPK pathway, leading to reactive oxidative species, causing cell death. Brain insulin resistance will also lead to senescent (halted cell cycle due to damaging DNA signals) glial cells, which produce neurotoxic pro-inflammatory cytokines (Erol, 2010).

To measure neuroinflammation, this project will evaluate the presence of pro-inflammatory factors such as TNF-α and IL-6 in the 5xFAD mice. These cytokines are found in areas that are ridden with plaques (Bomfim et al., 2012). Consequently, cytokines such as TNF-α mediate cell death by inhibiting cell survival pathways such as PI3K (Bomfim et al., 2012).

Limitations of this project involved the mashed food and the exposure to food presentations. For mashed food, food spillage was always an issue to control. Consequently, food intake may have been overestimated, altering interpretation of results. Exposure to food presentations was problematic in that mice were generally exposed to food on hopper for the majority of their lifespan and given food in hopper and mashed food for one week each. Consequently, lack of effect for food presentation for either body weight or frailty may due to brief exposure to food presentations. Another issue is the crude measurements of body composition. A DEXA scanner (PIXImus2; Lunar, Madison, WI) would allow us to evaluate all WAT and lean muscle in mice giving us an accurate picture of body composition in the 5xFAD mice. Measuring the gastrocnemius muscle may not give us a holistic picture of lean muscle in this mouse model. Indeed, both AD patients and other mouse models, such as the Tg2576, exhibit lean muscle reductions (Ishii et al., 2014; Lowry, 2010).

4.5 Conclusion

This project has described the feeding behaviour, body weight, and activity in the 5xFAD mouse model. We have seen that regardless of food presentation (FOH, FIC, and M), food intake and body weight were lower in 5xFAD than WT mice starting at 9 months of age and continuing to 12 months of age. Frailty was higher in the 5xFAD mice

and remained unchanged after each food presentation. To try to explain weight loss behaviourally we measured the correlation between body weight and food intake and measured activity. We found that the strength of relationship between body weight and food intake was weak, indicating that other factors most likely influence body weight reduction. The 5xFAD mice were hypoactive and were more likely to exhibit catatonic behaviour. The study found that WAT was predominately reduced in the 5xFAD mice. We next measured whether markers for adiposity and adipogenesis were reduced and markers for thermogenesis were elevated. Indeed, leptin, UCP-2, and HSL were markedly reduced, indicating abnormal levels of neuroendocrine signaling between the brain and WAT. UCP-1 was elevated in 5xFAD mice indicating elevated thermogenesis and fat burn. Finally the project evaluated plasma insulin and glucose levels. No abnormalities were seen in levels of insulin and glucose however insulin resistance in the brain of these mice remains possible. To summarize, 5xFAD mice exhibit similar symptoms as AD patients in that they lose weight and appetite. They show reductions in leptin, much like AD patients. This reduction in weight is partially explained by reduced food intake, adipogenesis, and elevated thermogenesis.

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