Effect of a high fat diet on a mouse model of Alzheimer’s disease

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

2010

Elysse Knight
# List of contents

List of contents .................................................................................................................. 2
List of figures ...................................................................................................................... 7
List of tables ....................................................................................................................... 10
Abstract of thesis ............................................................................................................... 11
Declaration ........................................................................................................................ 12
Copyright statement ......................................................................................................... 12
List of abbreviations .......................................................................................................... 13
Acknowledgements ........................................................................................................... 15

Chapter 1 .......................................................................................................................... 16
  1.0. Overview ..................................................................................................................... 17
  1.1. Alzheimer’s disease (AD) ......................................................................................... 17
  1.2. Behavioural and cognitive decline in AD patients .................................................... 17
  1.3. AD pathology ............................................................................................................ 18
  1.4. Experimental models of AD .................................................................................... 20
    1.4.1. 3xTgAD model of AD ......................................................................................... 21
  1.5. Risk factors for AD ................................................................................................... 23
  1.6. Obesity and energy balance ...................................................................................... 23
  1.7. AD and energy balance ............................................................................................ 24
    1.7.1. Mid-life obesity and risk of AD ........................................................................... 24
    1.7.2. Later-life obesity and risk of AD ........................................................................ 25
    1.7.3. Diet and risk of AD ............................................................................................ 26
    1.7.4. Dietary modification in AD models ..................................................................... 27
      1.7.4.1. HF diets ......................................................................................................... 27
      1.7.4.2. Calorie restricted diets ............................................................................... 28
    1.7.5. Weight loss and AD ......................................................................................... 28
  1.8. Summary and PhD hypothesis ............................................................................... 31

Chapter 2 .......................................................................................................................... 32
  2.1. Animals ..................................................................................................................... 33
  2.2. Examination of energy balance .............................................................................. 33
    2.2.1. Diet ................................................................................................................... 33
    2.2.2. Longitudinal body weight and food intake analysis ............................................. 34
    2.2.3. Indirect calorimetric measurement of metabolic rate .......................................... 34
  2.3. Assessment of behaviour and memory ................................................................... 35
4.2.1. Experimental design .................................................................................................................................................. 70
  4.2.1.1. OF test .................................................................................................................................................................. 70
  4.2.1.2. SA test ............................................................................................................................................................... 70
  4.2.1.3. MWM task ......................................................................................................................................................... 70
  4.2.1.4. NOR test ............................................................................................................................................................ 71
  4.2.1.4. OR test ............................................................................................................................................................... 71
4.2.2. Statistical analysis ....................................................................................................................................................... 71
4.3. Results .................................................................................................................................................................................. 72
  4.3.1. Characterisation of behaviour and memory deficits in 3xTgAD mice ................................................................. 72
    4.3.1.1. 3xTgAD mice displayed age-dependent changes in open-field (OF) behaviour ........................................... 72
    4.3.1.2. 3xTgAD mice showed age-dependent memory deficits in the Y-maze spontaneous alternation (SA) test from 5 months of age ........................................................................................................ 73
    4.3.1.3. 3xTgAD mice had age-dependent memory deficits in the Morris water maze (MWM) task probe test from 5 months of age ........................................................................................................ 74
    4.3.1.4. 3xTgAD mice displayed age-dependent memory deficits in the novel object recognition (NOR) test from 8 months of age ................................................................................................... 76
    4.3.1.5. 3xTgAD mice showed memory deficits in odour recognition (OR) test at 3 months of age ......................... 77
4.3.2. Evaluation of behaviour and memory in 129SV and C57BL/J6 mouse strains ....................................................... 78
  4.3.2.1. 129SV and C57BL/J6 mice displayed no difference in open field (OF) behaviour at 3, 8, 12 and 18 months of age ..................................................................................................................... 78
  4.3.2.2. 129SV and C57BL/J6 mice showed no difference in memory in the Y-maze spontaneous alternation (SA) test at 3, 8, 12 and 18 months of age ........................................................................ 79
4.4. Discussion ............................................................................................................................................................................. 80
  4.4.1. Open-field (OF) behaviour test ................................................................................................................................. 80
    4.4.1.1. OF behavioural test conclusions and future studies .......................................................................................... 82
  4.4.2. Y-maze spontaneous alternation (SA) memory test ................................................................................................. 82
    4.4.2.1. Y-maze SA test conclusions and future studies .............................................................................................. 83
  4.4.3. Morris water maze (MWM) memory task .................................................................................................................. 83
    4.4.3.1. MWM task conclusions and future studies .............................................................................................. 85
  4.4.4. Novel object recognition (NOR) memory test ........................................................................................................... 85
    4.4.4.1. NOR test conclusions and future studies .............................................................................................. 86
  4.4.5. Odour recognition (OR) task ................................................................................................................................. 86
    4.4.5.1. OR test conclusions and future studies .............................................................................................. 87
  4.4.6. 129SV and C57BL/J6 strains .................................................................................................................................. 88
4.5. Conclusion .............................................................................................................................................................................. 88
Chapter 5 ................................................................................................................................................................................. 89
  5.1. Introduction and objectives .............................................................................................................................................. 90
  5.2. Materials and methods ................................................................................................................................................. 90
    5.2.1. Experimental design ............................................................................................................................................. 90
5.4. Discussion

5.3. Effect of an HF diet on energy balance in 3xTgAD mice

5.3.1. Effect of an HF diet on energy balance in 3xTgAD mice

5.3.1.1. Effect of an HF diet on body weight in 3xTgAD mice

5.3.1.2. Effect of an HF diet on energy balance and metabolic rate in 3-4, 7-8, 11-12 and 15-16-month-old 3xTgAD mice in CLAMS metabolic cages

5.3.1.2.1. Effect of an HF diet on body weight, caloric intake and water intake in 3-4-month-old 3xTgAD mice in CLAMS metabolic cages

5.3.1.2.2. Effect of an HF diet on metabolic rate in 3xTgAD mice at 3 months of age

5.3.1.2.3. Effect of an HF diet on body weight, caloric intake and water intake of 7-8-month-old 3xTgAD mice in CLAMS metabolic cages

5.3.1.2.4. Effect of an HF diet on metabolic rate in 3xTgAD mice at 7-8 months of age

5.3.1.2.5. Effect of an HF diet on body weight, caloric intake and water intake of 11-12-month-old 3xTgAD mice individually housed in CLAMS metabolic cages

5.3.1.2.6. Effect of an HF diet on metabolic rate in 3xTgAD mice at 11 months of age

5.3.1.2.7. Effect of an HF diet on metabolic rate in 3xTgAD mice at 16 months of age

5.3.1.2.8. Effect of an HF diet on metabolic rate in 3xTgAD mice at 16 months of age

5.3.2. Effect of an HF diet on behaviour and memory in 3xTgAD mice at 3-4, 7-8, 11-12 and 15-16 months of age

5.3.2.1. Effect of an HF diet on open-field (OF) behaviour in 3xTgAD mice at 3-4, 7-8, 11-12 and 15-16 months of age

5.3.2.2. Effect of an HF diet on Y-maze spontaneous alternation (SA) in 3xTgAD mice at 3-4, 7-8, 11-12 and 15-16 months of age

5.3.2.3. Effect of an HF diet on the Morris water maze (MWM) task in 3xTgAD mice at 3-4, 7-8, 11-12 and 15-16 months of age

5.3.2.4. Effect of an HF diet on memory in the novel object recognition (NOR) test in 3xTgAD mice at 3-4, 7-8, 11-12 and 15-16 months of age

5.3.2.5. Effect of an HF diet on memory in the odour recognition (OR) test in 3xTgAD mice at 3-4, 7-8, 11-12 and 15-16 months of age

5.4. Discussion

5.4.1. Effect of an HF diet on energy balance in 3xTgAD mice

5.4.1.1. Energy balance of control 3xTgAD mice on a chow diet

5.4.1.2. Effect of an HF diet on energy balance in 3xTgAD mice

5.4.1.3. Previous HF studies in mouse models of AD
5.4.1.4. Effect of an HF diet on energy balance summary ................................. 124
5.4.2. Effect of an HF diet on behaviour and memory in 3xTgAD mice .................. 125
  5.4.2.1. Behaviour and memory in control 3xTgAD mice on a chow diet ............... 125
  5.4.2.2. An HF diet induced memory deficits in both Non-Tg control and 3xTgAD mice .. 125
  5.4.2.3. The effect of an HF diet on behaviour and memory in models of AD .......... 126
  5.4.2.4. An HF diet affects memory in cognitively normal rodents and humans ........ 127
  5.4.2.5. Potential mechanisms underlying diet-induced memory deficits ............... 128
  5.4.2.6. Effect of an HF diet on energy balance and behaviour and memory summary... 130

Chapter 6 .................................................................................................................. 131
  6.1 Final discussion .................................................................................................. 132

Appendices ............................................................................................................... 136
 Appendix A: Nutritional profiles of chow and HF diets ........................................ 137
 Appendix B: Genotyping of 3xTgAD and Non-Tg control mice .............................. 139
 Appendix C: Published manuscript ....................................................................... 142

References ............................................................................................................... 150

Final word count: 45,851
List of figures

Chapter 1: Introduction

1.1. Post-mortem pathological hallmarks in the brain of an AD patient with visible amyloid plaques and neurofibrillary tau tangles (NFTs) .................................................................18
1.2. Formation of Aβ plaques and NFT tau tangles ........................................19
1.3. Schematic representation of the energy balance system...........................24

Chapter 2: General materials and methods

2.1. Comprehensive Laboratory Animal Monitoring System (CLAMS) ........34
2.2. Open-field (OF) test. ..............................................................................36
2.3. Intra-maze cues in the Y-maze spontaneous alternation (SA) test. ..........37
2.4. Y-maze spontaneous alternation (SA) test. ...........................................37
2.5. Time-line of the odour recognition (OR) test. .......................................39
2.6. Schematic representation of the odour recognition (OR) test. .................40
2.7. Time-line of the novel object recognition (NOR) test. .........................41
2.8. Schematic representation of the Novel Object Recognition (NOR) test. ...42
2.9. Set-up of the Morris water maze (MWM) ............................................44
2.10. Extra maze spatial cues in the Morris water maze (MWM) ....................45
2.11. Time-line of the Morris water maze (MWM) task ..............................46

Chapter 3: Characterisation of energy balance in 3xTgAD mice

3.1. 2-month-old male 3xTgAD mice displayed increased body weight and food intake ....53
3.2. 3xTgAD mice had altered body weight and food intake profiles ................54
3.3. Decreased survival in female 3xTgAD mice ..........................................55
3.4. 2-month-old 3xTgAD mice displayed no difference in metabolic rate .......56
3.5. 12-month-old 3xTgAD mice had a higher metabolic rate .......................58
3.6. 18-month-old 3xTgAD mice had a higher metabolic rate .........................60
3.7. Male 129SV and C57BL/J6 mice displayed similar body weight and food intake profiles over 20 months .................................................................61
3.8. 20-month-old male 129SV and C57BL/J6 mice displayed similar body weight, food intake, water intake and metabolic rate ........................................62

Chapter 4: Characterisation of behaviour and memory in 3xTgAD mice

4.1. 3xTgAD mice displayed age-dependent changes in open-field (OF) behaviour ..........72
4.2. 3xTgAD mice showed age-dependent memory deficits in the Y-maze spontaneous alternation (SA) test from 5 months of age ................................................................. 73
4.3. 3xTgAD mice had age-dependent memory deficits in the Morris water maze (MWM) probe test from 5 months of age ................................................................. 75
4.4. 3xTgAD mice showed age-dependent memory deficits in the novel object recognition (NOR) test from 8 months of age ................................................................. 76
4.5. 3-month-old male 3xTgAD mice displayed memory deficits in the odour recognition (OR) test ........................................................................................................ 77
4.6. 129SV and C57BL/J6 mice had no difference in open-field (OF) behaviour at 3, 8, 12 and 18 months of age ................................................................. 78
4.7. 129SV and C57BL/J6 had no difference in memory in the Y-maze spontaneous alternation (SA) test/ at 3, 8, 12 and 18 months of age ................................................................. 79

Chapter 5: Effect of a high fat diet on 3xTgAD mice

5.1. Effect of a high fat (HF) diet on body weight in 3xTgAD mice at 3-4, 7-8, 11-12 and 15-16 months of age ................................................................. 93
5.2. Effect of a high fat (HF) diet on body weight, caloric intake and water intake in individually housed 3-4-month-old 3xTgAD mice in Comprehensive Laboratory Animal Monitoring System (CLAMS) cages ................................................................. 95
5.3. Effect of a high fat (HF) diet on metabolic rate in 3-4-month-old 3xTgAD mice ................................................................. 97
5.4. Effect of a high fat (HF) diet on body weight, caloric intake and water intake in individually housed 7-8-month-old 3xTgAD mice in Comprehensive Laboratory Animal Monitoring System (CLAMS) cages ................................................................. 99
5.5. Effect of a high fat (HF) diet on metabolic rate in 7-8-month-old 3xTgAD mice ................................................................. 101
5.6. Effect of a high fat (HF) diet on body weight, caloric intake and water intake in 11-12-month-old 3xTgAD mice individually housed in CLAMS metabolic cages ................................................................. 103
5.7. Effect of a high fat (HF) diet on metabolic rate in 11-12-month-old 3xTgAD mice ................................................................. 105
5.8. Effect of a high fat (HF) diet on body weight, caloric intake and water intake in 15-16-month-old 3xTgAD mice individually housed in CLAMS metabolic cages ................................................................. 107
5.9. Effect of a high fat (HF) diet on metabolic rate in 15-16-month-old 3xTgAD mice ................................................................. 109
5.10. Effect of high fat (HF) diet on epididymal fat pad weight in 3xTgAD mice at 3-4, 7-8, 11-12 and 15-16 months of age ................................................................. 111
5.11. Effect of high fat (HF) diet on open-field (OF) behaviour in 3xTgAD mice at 3-4, 7-8, 11-12 and 15-16 months of age ................................................................. 113
5.12. Effect of a high fat (HF) diet on Y-maze spontaneous alternation (SA) performance in 3xTgAD mice at 3-4, 7-8, 11-12 and 15-16 months of age ................................................................. 115
5.13. Effect of a high fat (HF) diet on the Morris water maze (MWM) task in 3xTgAD mice at 3-4, 7-8, 11-12 and 15-16 months of age ................................................................. 117
5.14. Effect of a high fat (HF) diet on memory in novel object recognition (NOR) test in 3xTgAD mice in 3-4, 7-8, 11-12 and 15-16 months of age .................................................................118
5.15. Effect of a high fat (HF) diet on memory deficits in odour recognition (OR) test in 3xTgAD mice at 3-4, 7-8, 11-12 and 15-16 months of age .................................................................120
5.16. Schematic representation of potential molecular pathways linking obesity and cognitive decline .................................................................................................................129

Appendices

B1. Genotyping of 3xTgAD and Non-Tg control mice .........................................................141
List of tables

Chapter 2: General materials and methods

2.1. Diet composition of chow and high fat diets .................................................................33
2.2. Scent combinations of the odour recognition (OR) task..................................................39
2.3. Flagged platform training trial pattern of the Morris water maze (MWM) test................47
2.4. Morris water maze (MWM) submerged platform training and probe trial start position pattern..................................................................................................................48

Chapter 3: Characterisation of energy balance in 3xTgAD mice

3.1. Experimental cohorts for energy balance assessment (comprehensive laboratory animal monitoring system; CLAMS).................................................................51

Chapter 5: Effect of a high fat diet on 3xTgAD mice

5.1. Experimental cohorts for energy balance assessment.......................................................90
5.2. Experimental cohorts for behavioural evaluation.............................................................91

Chapter 6: Final discussion

6.1. Summary of the characterisation of energy balance in 3xTgAD mice versus Non-Tg control mice................................................................................................................132
6.2. Summary of the characterisation of behaviour and memory in 3xTgAD mice versus Non-Tg control mice........................................................................................................133
6.3. Summary of the effect of a high fat (HF) diet on a battery of cognitive tests in 3xTgAD and Non-Tg control mice........................................................................................................133
Alzheimer’s disease (AD) is a progressive neurodegenerative disorder, characterised by deficits in language, behaviour and memory. Increasing evidence suggests that mid-life obesity and a diet high in fat are risk factors for AD. In contrast, life-threatening weight loss occurs and worsens as the disease progresses, despite adequate or increased food intake. A greater understanding of energy balance in AD may therefore uncover novel targets for therapy. The aim of this thesis was to test the hypothesis that 3xTgAD mice display altered energy balance and that experimental changes to this balance will alter cognition. To address this hypothesis, three key objectives were set up; to characterise the energy balance profile, characterise behaviour and memory, and evaluate the response to an high fat (HF) diet in a triple transgenic (3xTgAD) model, an experimental mouse model of AD.

Energy balance was characterised in non-transgenic (Non-Tg) control and 3xTgAD mice, demonstrating altered body weight, food intake and metabolic rate in the 3xTgAD mouse model of AD. At 2-month of age male 3xTgAD mice displayed greater food intake and body weight, but no difference in metabolic rate, whereas from 12 months of age 3xTgAD mice weighed less, despite eating more, and had a higher metabolic rate than Non-Tg control mice. This provides evidence that there is a shift towards a hypermetabolic state from 12 months of age in 3xTgAD mice, which may represent a key stage in advancement of the disease process. Behaviour and memory were characterised in Non-Tg control and 3xTgAD mice in a battery of tests at different ages. 3xTgAD mice showed changes in open-field activity/anxiety from 3 months of age. Memory impairments were first detected in 3xTgAD mice at 3 months of age as deficits in odour recognition memory, mirroring early impairments seen in AD patients. Deficits in spatial memory were then observed in both the Y-maze spontaneous alternation and Morris water maze tests from 5 months of age. Finally, deficits in non-spatial visual object memory were observed in 3xTgAD mice in the novel object recognition test at 8 months of age. Energy balance, behaviour and memory were assessed in Non-Tg control and 3xTgAD mice in response to an HF diet. Non-Tg control and 3xTgAD mice displayed similar energy balance profiles in response to an HF diet. The HF diet was found to worsen memory in Non-Tg mice in odour recognition at 3-4 and 7-8 months of age, in the Morris water maze at 7-8 months of age and in novel object recognition and spontaneous alternation at 11-12 and 15-16 months of age. Similarly, the HF diet worsened memory in 3xTgAD mice in odour discrimination at 3-4 and 7-8 months of age, the Morris water maze at 7-8 and 11-12 months of age, and in spontaneous alternation at 15-16 months of age. As an HF diet induced memory impairments, in both Non-Tg control and 3xTgAD mice, it suggests that diet-induced deficits may therefore, not be specific to AD, but rather to cognition in general.

Overall, these data demonstrate that 3xTgAD mice show AD-like age-dependent changes in energy balance, behaviour and memory. Furthermore, an HF diet produced impairments in memory in 3xTgAD mice, but these effects were not specific to AD, as an HF diet also led to deficits in control animals. These data support a role for energy balance in the progression of AD, although the underlying mechanisms remain poorly understood. 3xTgAD mice may therefore represent a good model to examine energy balance during AD and to evaluate targets for future therapies.
Declaration

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

Elysse Knight

Copyright statement

i. The author of this thesis (including any appendices and/or schedules to this thesis) owns certain copyright or related rights in it (the “Copyright”) and s/he has given The University of Manchester certain rights to use such Copyright, including for administrative purposes.

ii. Copies of this thesis, either in full or in extracts and whether in hard or electronic copy, may be made only in accordance with the Copyright, Designs and Patents Act 1988 (as amended) and regulations issued under it or, where appropriate, in accordance with licensing agreements which the University has from time to time. This page must form part of any such copies made.

iii. The ownership of certain Copyright, patents, designs, trade marks and other intellectual property (the “Intellectual Property”) and any reproductions of copyright works in the thesis, for example graphs and tables (“Reproductions”), which may be described in this thesis, may not be owned by the author and may be owned by third parties. Such Intellectual Property and Reproductions cannot and must not be made available for use without the prior written permission of the owner(s) of the relevant Intellectual Property and/or Reproductions.

iv. Further information on the conditions under which disclosure, publication and commercialisation of this thesis, the Copyright and any Intellectual Property and/or Reproductions described in it may take place is available in the University IP Policy (see http://www.campus.manchester.ac.uk/medialibrary/policies/intellectual-property.pdf), in any relevant Thesis restriction declarations deposited in the University Library, The University Library’s regulations (see http://www.manchester.ac.uk/library/aboutus/regulations) and in The University’s policy on presentation of Theses.
# List of abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Almond</td>
</tr>
<tr>
<td>Aβ</td>
<td>Amyloid-beta</td>
</tr>
<tr>
<td>AD</td>
<td>Alzheimer’s disease</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>APH-1</td>
<td>Anterior pharynx detective 1</td>
</tr>
<tr>
<td>ApoE</td>
<td>Apolipoprotein E</td>
</tr>
<tr>
<td>APP</td>
<td>Amyloid precursor protein</td>
</tr>
<tr>
<td>BACE</td>
<td>Beta-site amyloid precursor protein-cleaving enzyme</td>
</tr>
<tr>
<td>BDNF</td>
<td>Brain-derived neurotrophic factor</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>BPSD</td>
<td>Behavioural and psychological symptoms of dementia</td>
</tr>
<tr>
<td>CAA</td>
<td>Cerebral amyloid angiopathy</td>
</tr>
<tr>
<td>CDK5</td>
<td>Cyclin-dependent kinase 5</td>
</tr>
<tr>
<td>CHO</td>
<td>Carbohydrate</td>
</tr>
<tr>
<td>CLAMS</td>
<td>Comprehensive laboratory animal monitoring system</td>
</tr>
<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>CR</td>
<td>Caloric restriction</td>
</tr>
<tr>
<td>d</td>
<td>Day</td>
</tr>
<tr>
<td>DEPC</td>
<td>Diethylpyrocarbonate</td>
</tr>
<tr>
<td>DHA</td>
<td>Docosahexaenoic acid</td>
</tr>
<tr>
<td>EOFAD</td>
<td>Early-onset familial Alzheimer’s disease</td>
</tr>
<tr>
<td>GSK3</td>
<td>Glycogen synthase kinase 3</td>
</tr>
<tr>
<td>h</td>
<td>Hour</td>
</tr>
<tr>
<td>HF</td>
<td>High fat</td>
</tr>
<tr>
<td>HPA</td>
<td>Hypothalamic-pituitary-adrenal axis</td>
</tr>
<tr>
<td>IA</td>
<td>Inhibitory avoidance</td>
</tr>
<tr>
<td>IDE</td>
<td>Insulin degrading enzyme</td>
</tr>
<tr>
<td>IF</td>
<td>Intermittent fasting</td>
</tr>
<tr>
<td>IRS-1</td>
<td>Insulin receptor substrate-1</td>
</tr>
<tr>
<td>ITI</td>
<td>Intertrial interval</td>
</tr>
<tr>
<td>JNK</td>
<td>c-jun N-terminal kinase</td>
</tr>
<tr>
<td>L</td>
<td>Lemon</td>
</tr>
<tr>
<td>LOAD</td>
<td>Late-onset Alzheimer’s disease</td>
</tr>
<tr>
<td>LTP</td>
<td>Long-term potentiation</td>
</tr>
<tr>
<td>MCI</td>
<td>Mild cognitive impairments</td>
</tr>
<tr>
<td>Min</td>
<td>Minute</td>
</tr>
<tr>
<td>MWM</td>
<td>Morris water maze</td>
</tr>
<tr>
<td>Non-Tg</td>
<td>Non-transgenic</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>NOR</td>
<td>Novel object recognition</td>
</tr>
<tr>
<td>NFT</td>
<td>Neurofibrillary tangles</td>
</tr>
<tr>
<td>O</td>
<td>Orange</td>
</tr>
<tr>
<td>OF</td>
<td>Open-field</td>
</tr>
<tr>
<td>P13K</td>
<td>Phosphatidylinositol 3,4,5 triphosphate</td>
</tr>
<tr>
<td>PB</td>
<td>Phosphate buffer</td>
</tr>
<tr>
<td>PEN-1</td>
<td>Presenilin enhancer-1</td>
</tr>
<tr>
<td>PFA</td>
<td>Paraformaldehyde</td>
</tr>
<tr>
<td>PKB</td>
<td>Protein kinase B</td>
</tr>
<tr>
<td>PS-1</td>
<td>Presenilin-1</td>
</tr>
<tr>
<td>PS-2</td>
<td>Presenilin-2</td>
</tr>
<tr>
<td>PUFA</td>
<td>Polyunsaturated fatty acids</td>
</tr>
<tr>
<td>RQ</td>
<td>Respiratory quotient</td>
</tr>
<tr>
<td>s</td>
<td>Second</td>
</tr>
<tr>
<td>SA</td>
<td>Spontaneous alternation</td>
</tr>
<tr>
<td>SAD</td>
<td>Sagittal abdominal diameter</td>
</tr>
<tr>
<td>SD</td>
<td>Standard diet</td>
</tr>
<tr>
<td>SR</td>
<td>Smell recognition</td>
</tr>
<tr>
<td>Tg</td>
<td>Transgenic</td>
</tr>
<tr>
<td>V</td>
<td>Vanilla</td>
</tr>
<tr>
<td>VO$_2$</td>
<td>Volume of oxygen</td>
</tr>
<tr>
<td>VCO$_2$</td>
<td>Volume of carbon dioxide</td>
</tr>
<tr>
<td>3xTgAD</td>
<td>Triple transgenic mouse model of Alzheimer’s disease</td>
</tr>
</tbody>
</table>
Acknowledgements

This work was funded by the Medical Research Council.

I would to like to thank everyone who has helped and supported me throughout my PhD:

I am deeply grateful to my supervisors Catherine Lawrence and Stuart Allan, and my advisor Robert Lucas for their complete support and guidance during the last 3 years.

Cath, I feel honoured to be your first PhD student. Thank you for all your time, patience and advice and for being a fun roommate at conferences! I will also miss our chats about food during perfusing sessions!

Stuart, thank you for always making time for me and being such a positive person. How you balance work and home is truly inspirational.

I would especially like to thank Cath and Stuart for all their help during our long perfusing sessions!

I would like to thank everyone in the lab for all their help and support, especially Penny and Lorraine for keeping me smiling! I would like to thank Sarah Gumusgoz for her help, especially genotyping all the mice used during this PhD. I would especially like to acknowledge everyone in the University of Manchester animal unit, my second family, for all their help and care, for making sure I had eaten something on long behaviour days and for making sure I was still alive in my little behaviour room over the weekends. I would also like to thank the disability office for all their time and support over my university education.

I would like to thank Apollo for being the most amazing fiancé, for looking after me, supporting me, making endless cups of tea and for all the hugs and pep talks during thesis writing. I would not have been able to finish this PhD without you. Thank you! Finally I would like to thank my mummy and daddy whose endless support, encouragement and belief in me have helped me get to where I am today.
Chapter 1

Introduction
1.0. Overview

This introduction provides a brief overview of Alzheimer’s disease (AD) and the various transgenic mouse models created. It will then examine the role of obesity and diet as risk factors for AD and review the studies assessing the role of diet and obesity in models of AD. Finally, the hypothesis and aims of this PhD will be presented.

1.1. Alzheimer’s disease (AD)

AD is a progressive age-dependent neurodegenerative disorder characterised by language, memory and behavioural deficits. It is the most common cause of dementia in the elderly, currently affecting around 5% of those aged 65 or over (Hardy 2006). By 2050, it is estimated that 370 million people worldwide will be aged 80 or older and that over 50% of this population will develop AD (United Nations 1998). There are currently no treatments to cure or delay the onset of AD, therefore further understanding the complex aetiology and progression of AD is important in an ageing population (Knight 2007).

1.2. Behavioural and cognitive decline in AD patients

There are two main types of memory, declarative and non-declarative memory. Declarative memory can be broken down into semantic (the ability to learn facts and concepts) and episodic memory (recalling past events and experiences) and is dependent on the hippocampus-medial temporal lobe (MTL) and the diencephalon, whereas non-declarative memory (habits and skills) is related to striatum, motor cortex and cerebellum (Squire 2004; Squire & Zola-Morgan 1991; Thompson & Kim 1996).

In AD, one of the first signs of cognitive decline is the generalised disruption of declarative memory, including deficits in both semantic and episodic memory, which is often characterised by an abnormally rapid rate of forgetfulness (Artero et al 2003; Carlesimo & Oscar-Berman 1992; Morris 1996; Welsh et al 1992). Deficits in episodic memory are one of the best indicators of early AD compared to other forms of dementia and have been reported in the pre-clinical stage of the disease (Artero et al 2003; Chen et al 2000b; Hodges 2006; Morris 1996; Welsh et al 1992; White & Ruske 2002). Mild cognitive impairments (MCIs) in recognition performance and free recall tasks have been observed in patients when compared to age-matched controls up to six years prior to diagnosis (White & Ruske 2002). The ability to encode and store new declarative information is impaired in AD patients from an early clinical stage and as AD progresses, other cognitive deficits including disruptions in language, spatial orientation, attention and executive functions manifest (Billings et al 2005; Chertkow & Bub 1990; Hodges 2006; Lambon Ralph et al 2003; Morris 1996; Perry & Hodges 1999). In contrast, procedural memory (habits and skills) remains relatively
unaffected until the late stages of the disease (Billings et al 2005; Chertkow & Bub 1990; Hodges 2006; Knight 2007; Lambon Ralph et al 2003; Morris 1996; Perry & Hodges 1999).

AD is associated with various behavioural and psychological symptoms including depression, anxiety, apathy, irritability, aggression, disinhibition and reduced curiosity (Brodaty & Low 2003; Honig & Mayeux 2001; Levy et al 1999; Masterman 2003; Pugh et al 2007; Ritchie & Lovestone 2002). These all form part of the behavioural and psychological symptoms of dementia (BPSD), which are commonly seen in AD, in either the early or late stages of the disease, and are known to fluctuate throughout its progression (Cummings 2000; Daffner et al 1992; Spalletta et al 2004). Disturbances in sleep-wake patterns are also a common feature of the disease (Bliwise 1993; Bonanni et al 2005; Lee et al 2007; Montplaisir et al 1998) with AD patients displaying increased sleepiness during the day and increased wakefulness at night (Bonanni et al 2005). AD patients often exhibit a shift in their body clock, tending to wake up later in the day and going to sleep later than non-demented controls (Lee et al 2007). Similarly circadian shifts in eating patterns are observed in AD patients who have a tendency to have their biggest meal during breakfast and display increased preference for sweet food (Keene & Hope 1997b; Young 2001).

The ability of AD sufferers to perform everyday activities becomes increasingly impaired over time, and eventually leads to permanent dependence on caregivers. Only when behavioural and cognitive deficits disrupt normal social and occupational function is AD clinically diagnosed (Morris 1996), however, AD can only be definitively diagnosed following post-mortem histopathological examination of brain tissue (Small 1998; White & Ruske 2002).

1.3. AD pathology

The pathology of AD within the brain is characterised by extracellular amyloid-beta (Aβ) plaques and intracellular neurofibrillary tangles (NFTs) composed of hyperphosphorylated tau (see Figure 1.1.)

![Figure 1.1. Post-mortem pathological hallmarks in the brain of an AD patient with visible amyloid plaques and neurofibrillary tau tangles (NFTs)](http://www.alz.org/brain/overview.asp)

It is believed that the inability to remove and/or the overproduction of fibrillar Aβ causes AD via an accumulation and deposition of amyloid and the production of NFTs (Hardy & Higgins 1992; Knight 2007) (see Figure 1.2.). Together these pathological hallmarks are the trigger for the neural degeneration and cognitive decline observed in AD patients (Hardy et al 1998; Tanzi 2005). In AD, pathology can be seen in the entorhinal cortex, hippocampus, frontal cortex, basal forebrain, parietal lobe, occipital lobe, amygdala, locus coeruleus and raphe nucleus and other hallmarks include inflammation, cerebral amyloid angiopathy, neurophil threads, granulovacuolar degeneration, synaptic alterations, neuronal loss, Lewy and Hirano bodies (Esiri 1996; Tapiola 2001).

**Figure 1.2. Formation of Aβ plaques and NFT tau tangles**

Alzheimer’s disease is characterised by dense intracellular neurofibrillary tau tangles (NFTs) and extracellular amyloid-beta (Aβ) plaques within the brain. Aβ peptide aggregates are formed via cleavage of amyloid precursor protein (APP) by ß- and γ-secretases resulting in Aβ40 and Aβ42. It is Aβ42, which forms insoluble amyloid fibrils readily that aggregate to form Aβ plaques that are considered to be the toxic species heavily implicated in the pathogenesis of AD. Alternatively, APP can and is cleaved by α-secretase, which does not generate the toxic species of Aβ. In AD tau becomes hyperphosphorlated, unstable and fragments to cause NFTs (Schematic redrawn from: [http://www.nia.nih.gov/NR/rdonlyres/835CFCB2-6222-45CB-A8F0-E4AD70220257/0/progress_report_pg34high.jpg](http://www.nia.nih.gov/NR/rdonlyres/835CFCB2-6222-45CB-A8F0-E4AD70220257/0/progress_report_pg34high.jpg)).

Aβ, the major component of amyloid plaques is formed via proteolytic processing of amyloid precursor protein (APP), a type I transmembrane protein (Carlson 2003; LaFerla & Oddo 2005; Munter et al 2007; Shen & Kelleher 2007) (Figure 2). APP is first cleaved by ß-secretase then by γ-secretase (Carlson 2003; Gotz et al 2006; LaFerla & Oddo 2005; Munter et al 2007; Shen &
Kelleher 2007). This process is known as the amyloidogenic pathway, in which the activity of β-secretase is attributed to β-site amyloid precursor protein-cleaving enzyme (BACE), whereas γ-secretase activity is dependent upon presenilin (PS), anterior pharynx defective 1 (APH-1), presenilin enhancer 1 (PEN-1) and nicastrin (Gotz et al 2006). Cleavage via β- and γ-secretases results in a short (Aβ40) and long form (Aβ42) (Oakley et al 2006) of Aβ. Aβ42 forms insoluble amyloid fibrils readily and is considered the toxic species heavily implicated in the pathogenesis of AD. Studies have revealed that an increased production of insoluble Aβ42 is caused by an alteration in APP metabolism (Hardy et al 1998; Oakley et al 2006). Furthermore, patients with inherited mutations in APP and PS-1 display increased levels of toxic Aβ42 (Munter et al 2007). In the normal ageing brain, APP is cleaved by α-secretase (disintegrin and metalloproteinase – ADAM - 10), which does not generate the toxic species of Aβ. This is known as the non-amyloidogenic pathway (Gotz et al 2006). In its normal state tau, a soluble protein, promotes microtubule assembly and stabilisation (LaFerla & Oddo 2005). There is no genetic link between AD and the tau gene, suggesting that tau lies downstream of Aβ. However, it remains unknown whether pathology and potential effects of tau are dependent or independent of Aβ (LaFerla & Oddo 2005).

There are two forms of AD, familial (rare) and non-familial (common). Early-onset familial AD (EOFAD) accounts for less than 5% of all AD cases with onset usually under 65 years of age (Bertram 2007). It is caused by autosomal dominant transmission of rare, yet highly penetrant, genetic mutations in presenilin (PS-1, PS-2) and APP (Hardy et al 1998; Tanzi 2005), however, the vast majority of cases occur after 65 years of age (late-onset AD; LOAD) and are termed “sporadic”. The aetiology of these “sporadic” cases is unknown, although increasing evidence suggests that genetic factors could be involved (Bertram 2007; Lippa et al 1996). Apolipoprotein-E (ApoE) has been shown to be a risk factor gene for AD, while both familial and sporadic forms of AD are associated with the allelic variation ε4 (Luchsinger & Mayeux 2004; Mayeux et al 1993). More recently the Genome-Wide Association Studies (GWAS) identified three new genes; clusterin (CLU), complement (CRI) and PICALM linked with increased risk of LOAD (Harold et al 2009; Lambert et al 2009). It is important to note that the pathological hallmarks of AD are indistinguishable between EOFAD and LOAD cases (Gotz et al 2006).

1.4. Experimental models of AD

As mechanisms underlying age-related neurodegeneration and cognitive decline in AD remain elusive (due to the reliance on post-mortem tissue), various animal models have been created (Duff & Suleman 2004; Hardy et al 1998). Murine models have successfully replicated some AD-like characteristics and develop age-dependent pathology, but thus far no model has displayed all the pathological and behavioural characteristics observed in humans (Duff & Suleman 2004; Oddo et al 2003a). This could be due to the role of environment and diet, amongst other factors, which
experimental models may not be exposed to, but also due to the complex nature of cognitive symptoms observed in humans, which do not have their obvious equivalent in animal models (Duff & Suleman 2004; Knight 2007; Oddo et al 2003a).

Gene-targeted and transgenic mice (Tg) have been created with mutant APP (Hsiao et al 1996), PS-1 (Mehta et al 1998), PS-2 (Herreman et al 1999), tau (Lewis et al 2001) and ApoE (Garcia-Alloza et al 2006; Oddo et al 2003a; Raber et al 1998). APP, PS-1, and PS-2 models are based on the overproduction of Aβ (due to overexpression of the aforementioned mutant human AD genes) and most develop age-dependent pathology (Duff & Suleman 2004). Mice overexpressing the mutant isoform of APP, PS-1 or PS-2 display Aβ plaques, while mutant tau mice show NFTs (Mandavilli 2006). Compared to the single transgenics, APP/PS-1 (Holcomb et al 1998) and APP/PS-2 (Richards et al 2003) double transgenics display accelerated accumulation and deposition of Aβ, but no NFTs. Transgenic models of AD also display behavioural and learning deficits in various tasks, including basal behaviour (open field activity), motor activity (balance beam, string agility), learning and memory (Y-maze spontaneous alternation, object recognition, holeboard, elevated plus-maze, Morris water maze (MWM), circular platform and radial arm maze) (Arendash et al 2001; Chapman et al 1999; Dodart et al 1999; Gotz & Ittner 2008; Hsiao et al 1996; King & Arendash 2002; King et al 1999; Savonenko et al 2005). These tasks are widely used in neuroscience and can examine various brain region specific aspects of learning and memory. More specifically to AD, these tasks have revealed that transgenic models of AD mice are born behaviourally normal and develop age-dependent deficits, with the age of onset varying between 3 and 6 months of age, depending on the task and experimental mouse model of AD used (King & Arendash 2002). Most authors have only examined one or two cognitive tasks in any one study, with the exception of Arendash and King (Arendash & King 2002) who examined intra- and inter-task relationships using a comprehensive battery of tasks (King & Arendash 2002).

1.4.1. 3xTgAD model of AD

A triple transgenic mouse model (3xTgAD) was developed by Oddo et al in 2003, which expresses mutant APP, PS-1 and tau. These mice display behavioural deficits and develop age-depandant Aβ plaque and tangle pathology, allowing the relationship between synaptic dysfunction, tangles and Aβ to be investigated (Oddo et al 2003b). Intraneuronal Aβ is first detectable in neurones of the neocortex of 3xTgAD mice, between 3-4 months of age, followed by CA1 then CA3 pyramidal neurones of the hippocampus by 6 months (Oddo et al 2003b). This is followed by an age-dependent progression in Aβ pathology, most of which is Aβ42, the toxic species associated with cognitive deficits. Intraneuronal Aβ is also visible within the amygdala. Extracellular Aβ plaques are first observed in 3xTgAD mice at 6 months of age within layers 4 and 5 of the neocortex. Interestingly, intraneuronal Aβ deposition precedes NFT formation, which is only detectable at 12-months (Oddo et al 2003a), supporting previous suggestions that tau is downstream of Aβ (LaFerla
Our preliminary data found age-dependent intracellular Aβ pathology in layers 4 and 5 of the neocortex, CA1 and CA3 pyramidal neurones of the hippocampus and the amygdala from as early as 1-2 months of age (Knight 2007; Rodriguez et al 2008). More recently we have observed that plaques are not visible until approximately 12 months of age and tau even later (Knight et al 2010). This is much later than originally reported by Oddo et al (2003b).

3xTgAD mice also develop deficits in synaptic plasticity, including long-term potentiation (LTP), the long-lasting increase in synaptic transmission, which is considered one of the major molecular mechanisms underlying learning and memory (Bliss & Collingridge 1993; Cooke & Bliss 2006; Oddo et al 2003b). Deficits in synaptic plasticity and LTP in 3xTgAD mice are associated with intracellular Aβ immunoreactivity, and appear prior to extracellular Aβ plaques and tangle deposition (Oddo et al 2003b). Such deficits in LTP are not observed in many other transgenic models of AD (Oddo et al 2003b), providing further evidence for the relevance of the 3xTgAD model to the human condition of AD.

3xTgAD mice display behavioural and cognitive deficits in sensorimotor function (wire rod and wire hang tests), spontaneous and circadian activity (open-field, plus maze, corner index measure, white-black box, dark-light maze tests), learning and memory (MWM) (Billings et al 2005). Billings et al (2005) determined that the initial cognitive impairments manifest as a deficit in retention, which correlates with the accumulation of intraneuronal Aβ (Billings et al 2005). The authors assessed cognitive deficits using the cued version of the MWM task, which is dependent on the hippocampus and inhibitory avoidance (IA), which is reliant on both the hippocampus and the amygdala. The authors found no impairments in the MWM at 2 months of age, when no overt Aβ and tau pathology has been previously reported. At 4 months, some behavioural deficits are observed, which are accompanied with some intraneuronal Aβ. However no extracellular Aβ plaques were seen at this stage. By 6 months of age, when mice have diffusible Aβ plaques in the cortex and intraneuronal Aβ in pyramidal neurones of the hippocampus, cortex and amygdala, mice were able to learn, but required more training than Non-Tg control mice. Contextual fear conditioning was also impaired at 6 months of age in 3xTgAD mice. These findings indicate that behavioural deficits progress in an age-dependent manner, the onset of which precedes plaque and tangle formation (LaFerla & Oddo 2005). In addition, these cognitive impairments can be rescued via clearance of intraneuronal Aβ with intracerebroventricular injection of anti- Aβ antibody (Billings et al 2005).

Experimental models have allowed detailed examination of the complex mechanisms involved in the pathogenesis of AD, and have revealed a wealth of information about the various factors that affect and influence the incidence of AD.
1.5. Risk factors for AD

Various risk factors have been implicated in the incidence of AD including age (advanced), Down’s syndrome, injury (head), sex (female), education (low), atherosclerosis (cardiovascular disease), susceptibility gene mutations (APP, PS-1, PS-2, ApoE) and environmental factors (diet, toxins, aluminium and viruses) (Thomas 2001). Emerging evidence suggests that obesity, an increasing health problem in developed societies, may also be a risk factor for AD (Gustafson et al 2003; Kivipelto et al 2005; Rosengren et al 2005; Whitmer et al 2005; Whitmer et al 2007). It is widely believed that the incidence of AD could be halved if the disease onset could be delayed by five years, therefore modification of factors such as diet could prove a crucial strategy for AD management (Pasinetti et al 2007).

1.6. Obesity and energy balance

Defined by an excess accumulation of adipose tissue, obesity (a body mass index; BMI ≥ 30 kg m$^{-2}$) is now considered a worldwide epidemic and is associated with many chronic diseases including cardiovascular disease, hypertension, hypercholesterolemia, insulin resistance, type II diabetes, arthritis, asthma, several types of cancer and low-grade inflammation (Mokdad et al 2003; Naderali et al 2009).

Body weight is regulated by the energy balance system (see Figure 1.3.), which controls the amount of food eaten and energy expended. Energy expenditure is composed of three components that include physical activity, diet-induced thermogenesis and basal metabolic rate (Levine 2005). Basal metabolic rate is the energy expended by an individual at rest, for normal cell and organ function within the body, and accounts for approximately 60% of total daily energy expenditure in individuals with a sedentary occupation (Joosen & Westerterp 2006; Levine 2005). Diet-induced thermogenesis accounts for approximately 10% of the total daily energy expenditure and is the thermic effect of food associated with digestion, absorption and storage of food. Physical activity or activity-induced thermogenesis is the most variable component from person to person, with individuals who exercise regularly it can account for up to approximately 10% of the total daily energy expenditure. There is also the activity of daily living, including maintaining posture when not recumbent, fidgeting and spontaneous muscle contraction. In most people this accounts for the rest of the total daily energy expenditure. There are also energy costs in response to altered temperature, medication and emotion which can effect total daily energy expenditure (Levine 2005).

The majority of obesity is associated with decreased levels of physical activity and increased consumption of sugar and saturated fats, causing an imbalance between caloric intake and energy expenditure (Spiegelman & Flier 2001). It is only when there is a positive energy balance, when
caloric intake is more than energy expenditure, that energy is stored in the form of triglycerides in adipose tissue (Spiegelman & Flier 2001).

Emerging reports suggest that obesity and poor diet are associated with increased risk of AD. Additionally, there appears to be a bimodal effect of adiposity on cognition, as during mid-life obesity increases risk of AD, whereas during late-life both increased BMI and being underweight are associated with increased risk. However, there is conflicting evidence that a high BMI late in life can reduce the risk of AD. This evidence will be reviewed in the next section.

1.7.1. Mid-life obesity and risk of AD

During mid-life (defined as approximately between 40 and 60 years of age (Friedland et al 2001)), evidence suggests that obesity is a risk factor for AD later in life (Fitzpatrick et al 2009; Rosengren et al 2005; Whitmer et al 2005; Whitmer et al 2008). At mid-life, a BMI between 20 and 22 is associated with the lowest risk of AD later in life, whereas a BMI over 30 was associated with a linear increase in risk (Rosengren et al 2005). Rosengren and colleagues also describe a BMI of less than 20 being associated with increased risk of AD later in life, although this was not significant. However, this study has been criticised as patients diagnosed in their early follow up period were not excluded (Gorospe & Dave 2007; Wang 2002). This is particularly important as
studies have shown that dementia patients that were previously obese can lose up to 50% of their body weight pre-dementia (Gorospe & Dave 2007; Wang 2002). The importance and aetiology of weight loss in AD will be discussed in more detail later.

The relationship between mid-life BMI and risk of future dementia was also examined by Kivipelto et al (2005), in a study with an average follow-up of 21 years, finding increased risk of AD later in life in participants with mid-life obesity (Kivipelto et al 2005). More recently, Fitzpatrick et al (2009) performed a prospective study on 2798 adults without dementia over a 4.5-year follow-up period, finding probable AD in 245 individuals. The study found increased risk of dementia in obese individuals (BMI > 30) versus those with a normal weight (BMI 20-25) at mid-life (Fitzpatrick et al 2009). Similarly, Whitmer et al (2005) completed a 27-year longitudinal population based study and found that mid-life obesity increased the risk of dementia later in life. In this study, obese patients had a 74% increased risk of developing dementia, while overweight patients had a 35% greater risk of dementia, compared to those within the normal weight range as defined by BMI and skin-fold thickness (Whitmer et al 2005). This was the first study to examine the risk of dementia in relation to skin-fold thickness (taken in the scapular and tricep region) and mid-life adiposity. Unfortunately, no data relating to nutrition, dieting or weight cycling was recorded (Whitmer et al 2005). More recently, the same authors examined the relationship between central obesity and risk of dementia later in life. It is important to distinguish central obesity (or “apple-shaped” obesity where fat is deposited around the abdomen) from peripheral obesity (or “pear-shaped” obesity where fat is deposited around the hips and thighs) (Rexrode et al 2001; Whitmer et al 2005), as it is central obesity that is highly associated with cardiovascular disease, insulin resistance, increased inflammatory markers and adipokines, all of which are suggested to play a role in dementia (Whitmer et al 2005). Whitmer et al (2008) examined sagittal abdominal diameter (SAD) as a measure of central obesity during mid-life (Whitmer et al 2008). The authors found that increased SAD significantly increased the risk of dementia three decades later in life. Participants with high SAD (> 25 cm) during mid-life had nearly a threefold increase in risk of dementia compared to those with the lowest SAD. Those participants with high SAD and high BMI were most at risk. Interestingly, participants with high SAD, yet normal BMI, displayed increased risk of dementia when compared to those with low SAD and normal BMI (Whitmer et al 2008). This study confirms the importance of taking physical measurements of obesity rather than relying on BMI, when examining the role of obesity in dementia.

1.7.2. Later-life obesity and risk of AD

The studies presented thus far provide evidence that mid-life obesity is associated with increased risk of AD in later life (> 65 years). The evidence regarding the effect of obesity on AD in later-life is, however, less clear.
Gustafson et al (2003) performed an 18-year follow-up study examining BMI and risk of AD and found that elderly participants who developed dementia had a higher BMI and were overweight, when compared to non-demented controls. Furthermore, at 70 years of age, participants displayed a 36% increased risk of AD for every annual unit increase in BMI (Gustafson et al 2003). In contrast, Buchman et al (2005) determined that declining BMI later in life was associated with increased risk of AD. Every annual unit decrease in BMI lead to a 35% increase risk of AD (Buchman et al 2005). The authors suggest that this decline in BMI may reflect ongoing pathological processes contributing to the development of AD (Buchman et al 2005). More recently, Razay et al (2006) examined the relationship between late-life BMI, waist-hip ratio and AD, finding a significant association with being obese or underweight (Razay et al 2006). This U-shaped association between later-life BMI and dementia has been supported by a meta-analysis study of 10 prospective cohorts looking at BMI, skin-fold thickness and waist circumference (Beydoun et al 2008). More recently, in a prospective study of 2798 participants, being underweight (BMI < 20) at later-life was associated with increased risk of AD, whereas obesity (> 35-30) was associated with a reduced risk when compared with participants with a normal BMI (BMI = 20-30) (Fitzpatrick et al 2009). Similarly, another prospective study in a cohort of 1836 patients, with a mean age over 71 years, with an average follow-up of 7 years observed higher BMI was associated with reduced risk of AD (Hughes et al 2009). In contrast to previous reports, these studies appear to indicate that late-life obesity has a protective effect on cognition (Naderali et al 2009).

The evidence presented thus far demonstrates that mid-life obesity increases the risk of future AD, whereas later in life both increased adiposity and being underweight are associated with increased risk, but this is not consistent, as some studies have shown that a high late-life BMI has a protective effect on cognition.

1.7.3. Diet and risk of AD

The majority of obesity is due to increases in food intake and usually an increase in fat consumption within the diet. Studies now show that high calorie or high fat (HF) diets affect risk of AD (Luchsinger & Mayeux 2004). A western diet (HF, high calorie) has been shown to be a high risk factor in the prevalence of AD (Holden 1999). Furthermore, elevated mid-life levels of cholesterol (usually observed in obese humans and individuals consuming an HF diet) have also been identified as a risk factor for AD (Pappolla et al 2003) and have been shown to be correlated with Aβ deposition (Kivipelto et al 2002). Both lipid metabolism and modulation of cholesterol in response to fat intake are related to ApoE, a known risk factor gene for AD (Berglund 2001; Luchsinger et al 2002; Petot et al 2003). High cholesterol levels are observed in people at high risk of AD with the ApoE4 allele, whereas those with the ApoE2 allele are at low risk of AD and have lower levels of cholesterol (Berglund 2001; Luchsinger & Mayeux 2004). Luchsinger et al (2002) completed a cohort study of 980 elderly people ≥ 65 years of age over a 4 year period finding that
those who possessed the ApoE ε 4 allele were in the highest quartile of calorie intake and total fat and had a high risk of AD (Luchsinger et al 2002). Other studies have demonstrated that regardless of ApoE genotype, high levels of trans-unsaturated and saturated fatty acids are associated with high risk of AD and associated cognitive decline (Luchsinger & Mayeux 2004). More recently, studies have attempted to look at dietary patterns in AD finding reduced risk being associated with decreased consumption of red meat, organ meat and butter (Gu et al 2010). Similarly another study demonstrated that AD is associated with increased consumption of meat, refined sugar, high-fat dairy products, butter and eggs (Gustaw-Rothenberg 2009).

In summary, the evidence presented to date implicates obesity and an HF/high cholesterol diet as risk factors for AD. However, the mechanisms underlying these associations of obesity and diet on AD remain poorly understood. Emerging evidence also suggests that environmental factors, especially nutrition, act in early life to program the risks for the onset of cardiovascular and metabolic disease in adult life (Alexe et al 2006; Holt & Byrne 2002; Morley 2006). However, it is not clear how changes in diet throughout life affect the onset or progression of AD. Even though AD is associated with old age, it is now known that the onset occurs years before any clinical symptoms manifest. This makes research into diet an important area of study, as it is an easily modifiable intervention, which may play a role in the onset and/or progression of AD.

1.7.4. Dietary modification in AD models

Increased caloric intake, especially saturated fats and obesity, are linked with higher incidence of AD in humans. These findings have been confirmed in work done in experimental models of AD, where animals maintained on a high fat diet display enhanced pathology and memory deficits.

1.7.4.1. HF diets

Studies in AD mouse models, demonstrate that mice fed an HF or high cholesterol diet, gain weight show increased AD-related pathology (George et al 2004; Ho et al 2004; Kohjima et al 2010; Levin-Allerhand et al 2002; Pedrini et al 2009; Refolo et al 2000; Shie et al 2002) and enhanced memory impairments compared to chow fed controls (Ho et al 2004). Ho et al (2004) examined the effect of an HF diet in female APP mice, finding that by 9 months of age HF fed APP mice developed insulin resistance, increased Aβ pathology within the neocortex and hippocampus of the brain, and impaired spatial learning in the MWM task (Ho et al 2004). To date, only one study has examined the effect of an HF diet on energy balance in 3xTgAD mice. When fed a 60% HF diet between 4-13 months of age, Non-Tg mice control gained more weight compared to respective controls on a chow diet, whereas the body weight of 3xTgAD mice was not significantly higher on HF diet. Despite this, HF fed 3xTgAD mice showed an increase in cortical Aβ and tau levels (Julien et al 2008). Very few studies have looked at both pathology and behaviour in response to an HF diet in
any model of AD. To date, in 3xTgAD mice, only pathology has been investigated in response to an HF diet, while the effect on behaviour is unknown.

1.7.4.2. Calorie restricted diets

In contrast to HF diets, studies have demonstrated that caloric restriction (CR) reduces Aβ pathology within the brain. Patel et al (2005) examined CR in two transgenic models of AD, male mice overexpressing APP, and double transgenic APP and PS-1 mice, and found a 40% and 55% reduction respectively in Aβ plaque pathology following CR (Patel et al 2005). Mice on the CR diet were given 60% of the food eaten by the ad-libitum fed mice. Similarly, Wang et al (2005) found amyloid pathology was almost completely attenuated in the neocortex and hippocampus of female Tg2576 mice placed on a CR diet, based on 70% of the average daily caloric intake of ad-libitum mice for nine months. The authors found improved glucose intolerance and a 3-fold lower fat pad weight in the CR animals (Wang et al 2005). Neither of the above studies examined the effect of CR on behavioural and memory deficits and no control Non-tg mice were used.

Halogappa et al (2007) examined the effect of 40% CR or intermittent fasting (IF) on behaviour and pathology in 3xTgAD mice. Male and female 3xTgAD mice were placed on a feeding regime at 3 months of age and open field activity and the MWM task were assessed up to 14 months of age. At this point, pathology was assessed by analysing Aβ1-40, Aβ1-42, tau and phospho-tau. The authors found that at 14 months of age CR and IF increased the reduction in locomotor activity and improved performance in the MWM task. Accordingly, Aβ40 and Aβ 42 were lower following CR in 3xTgAD mice, although this was not seen with IF. CR also reduced phospho-tau levels. The authors suggest that IF may prevent synapses from being damaged by Aβ and that dietary effects may suppress AD via upstream or downstream regulation of Aβ (Halogappa et al 2007).

1.7.5. Weight loss and AD

Although obesity has been identified as a risk-factor for AD, weight loss is a common feature of the disease, affecting between 30 and 40% of patients with mild to moderate AD (Gillette Guyonnet et al 2007; Gillette-Guyonnet et al 2000; Guerin et al 2005; White et al 1996). Furthermore, the risk of weight loss increases with severity and progression of AD (White et al 1998) and has been shown to be a predictor of mortality (Pugh et al 2007). In a geriatric population, weight loss of 4% or more in one year significantly correlates with increased risk of mortality (Wallace et al 1995). Guyonnet et al (1998) followed a cohort of 76 home-living patients with mild to moderate AD, finding that during the follow-up year, 45% of patients displayed a weight loss of 4% or more (Guyonnet 1998).

The reasons for this life-threatening weight loss are currently unknown. In order to lose lean or fat mass, energy intake must not be adequate to meet body requirements. It has been suggested that
the weight loss may be due to a reduction in dietary energy intake, as AD patients may have problems accessing food, or feeding themselves, or caregivers may have lack of information regarding nutrition or be too exhausted to provide adequate care (Gillette-Guyonnet et al 2000). However, food intake in individuals with AD is not usually lower, but in fact is adequate for their body weight, or even increased (Burns et al 1989; Keene & Hope 1997a; b; Niskanen et al 1993; Singh et al 1988; Smith et al 1999; Spindler et al 1996). Singh et al (1988) observed that AD patients weighed approximately 21% less than age-matched cognitively normal controls, yet no deficits in food intake or malabsorption were detectable (Singh et al 1988). Burns et al (1988) observed lower BMI, mid-arm circumference, arm muscle bulk and skin-fold thickness in patients with dementia, yet they displayed a higher energy needs (Burns et al 1989). Similarly, Spindler et al (1996) did a 1-year study looking at nutritional status, finding that AD patients had a significantly higher energy intake compared to aged-matched cognitively normal controls, yet their body weight was not significantly different. AD patients had significantly lower activity counts, measured using an accelerometer sewn into the clothes (Spindler et al 1996). More recently, weight loss, nutritional status and physical activity in AD patients were examined by Wang et al (2004) who observed significant weight loss in more than half of patients, which could not be explained, as those patients tended to consume more calories per kilogram of body weight per day. When food composition was analysed, the AD patients tended to eat more carbohydrates in their diet compared to controls (Wang et al 2004). Keene and Hope (1997) examined food intake and preference in dementia, finding that when food is not restricted, dementia patients display hyperphagia and an increased preference for sweet food (Keene & Hope 1997a; b).

The evidence presented thus far suggests that AD patients may have higher energy requirements than healthy individuals to maintain their body weight, and that when this energy demand is not met weight loss occurs, implying that energy expenditure is increased in AD. Energy expenditure is composed of three main factors, resting energy expenditure, diet-induced thermogenesis and energy of physical activity (van Baak 1999). Various studies have examined energy expenditure in AD patients and the results are contradictory, as no difference (Donaldson et al 1996; Niskanen et al 1993; Poehlman et al 1997), a decrease (Prentice et al 1989; Wang et al 1997) or an increase (Wolf-Klein et al 1995) in energy expenditure has been reported. Reasons for these inconsistencies are unknown, but maybe related to the severity and/or stage of the disease. Furthermore, in order to understand if an increase in energy expenditure is responsible for the weight loss observed in AD patients, measurements of energy expenditure during the dynamic phase of weight loss will be required.

AD appears to be associated with two problems in energy balance; mid-life obesity increases the risk of developing AD later in life, whereas life-threatening weight loss occurs and worsens as the disease progresses. Additionally, evidence suggests that weight loss may even precede clinical diagnosis of AD (Barrett-Connor et al 1996). Barrett-Connor et al examined body weight in a sample of community-dwelling people from three clinics from 1972 to 1993 (Barrett-Connor et al
1996). After three visits, patients were classified as being cognitively intact or having probable or possible AD. No weight loss was observed in cognitively intact individuals, whereas those that were later diagnosed with AD presented with significant weight loss after the baseline visit (Barrett-Connor et al 1996; Nourhashemi & Vellas 2008). These results have been mirrored in other studies, where accelerated weight loss was found to be a significant predictor of those individuals who converted from cognitively normal to AD (Johnson et al 2006; Nourhashemi & Vellas 2008). Weight loss may therefore reflect the disease process, with a shift in body weight representing an early indicator of AD, prior to clinical presentation. Mechanisms behind the weight loss are currently unknown, since food intake is usually adequate or even increased in AD patients. For this reason, further research into energy balance in AD is clearly of interest and may provide additional evidence underpinning the pathological mechanisms involved in AD.

The evidence presented thus far implicates obesity and a HF/high cholesterol diet as risk factors for AD. However, as this introduction has shown, obesity may actually be beneficial later in life as weight loss later in life can be life-threatening. It is clear that energy balance in AD is complicated and poorly understood, and requires further examination before diet related AD therapies can be used.
1.8. Summary and PhD hypothesis

There are many risk factors associated with AD, including an HF diet and obesity, observations which are supported by studies in both single and double transgenic models of AD where a high fat diet increases Aβ deposition within the brain and associated memory deficits. Despite this, the mechanisms underlying the effects of obesity and HF diets on AD are poorly understood. Furthermore, it remains unknown whether an HF diet enhances the onset and/or worsens the severity of AD-like pathology and memory deficits in 3xTgAD mice. Emerging evidence also suggests that environmental factors, especially nutrition, act in early life to program the risks for the onset of cardiovascular and metabolic disease in adult life. However, it is not clear how changes in diet throughout life affect the onset or progression of AD. Even though AD is associated with old age, it is now known that the onset occurs years before any clinical symptoms manifest, and so understanding the effect of environmental factors such as an HF diet throughout life is important in an increasingly obese and aging society.

While obesity increases the risk of developing the AD, life-threatening weight loss occurs and worsens as the disease progresses. Evidence suggests that weight loss may even precede diagnosis of AD (Barrett-Connor et al 1996), therefore reflecting the disease process, and a shift in body weight may represent an early indicator of AD prior to clinical presentation. Mechanisms behind the weight loss are currently unknown, since food intake is usually adequate or even increased in AD patients. For this reason, further research into energy balance in AD is clearly of interest and may provide further evidence underpinning the pathological mechanisms involved in AD. To date, very few studies have examined energy balance in transgenic models of AD.

The overall aim of this PhD project is to test the hypothesis that 3xTgAD mice have altered energy balance and that experimental changes to this balance will alter cognition.

To address this hypothesis the three main objectives of this PhD are to:

- characterise the energy balance profile of 3xTgAD mice
- characterise behaviour and memory in a battery of tests in 3xTgAD mice
- evaluate the effect of an HF diet on cognition in 3xTgAD mice

In order to examine these objectives, cohorts of 3xTgAD mice and age-matched Non-Tg controls will have their body weight and food intake measured longitudinally. If alterations in energy balance are detected, then metabolism will be investigated in an attempt to understand these changes. Behavioural and memory tests will then be established to determine whether 3xTgAD mice display deficits, with a view to see how they are affected if energy balance is changed experimentally using an HF diet.
Chapter 2

General materials and methods
2.1. Animals

3xTgAD mice expressing mutant PS1\textsubscript{M146V}, APP\textsubscript{Swe}, Tau\textsubscript{P301L} and non-transgenic (Non-Tg) C57BL/J6 x 129SV were created and supplied by Frank LaFerla (Irvine, CA) (Oddo et al 2003b). Male C57BL/J6 and 129SV mice, the individual strains of the hybrid C57BL/J6 x 129SV background on which the 3xTgAD and Non-Tg control mice were created, were purchased at 4 weeks of age from Harlan UK Ltd. (Bicester, Oxon, UK). The mice were kept in standard housing conditions (humidity 50-60 %, temperature 21± 1°C, 12:12 hour light-dark cycle with lights on at 8.00am) and given ad libitum access to standard rodent chow and water. They were also ear-punched for identification. All animal experiments were carried out in accordance with the animals (Scientific Procedures) Act 1986.

2.2. Examination of energy balance

In order to evaluate whether 3xTgAD mice display differences in energy balance when compared to Non-Tg control mice, body weight, food intake and metabolism were examined in cohorts of male and female 3xTgAD and Non-Tg controls. A separate cohort of male C57BL/J6 and 129SV mice, were also monitored to examine potential strain specific differences in body weight, food intake and metabolic rate.

2.2.1. Diet

Once weaned, at approximately 4 weeks of age, the mice were placed on standard laboratory chow (diet BK001E, B&K Universal LTD, UK). At 2 months of age the mice were then either kept on chow or placed on a 60 % high fat (HF) diet (diet 58G9, TestDiet, Richmond, IN, USA) (see Table 2.1.). See Appendix A for detailed nutritional profiles of both chow and HF diets.

Table 2.1. Diet composition of chow and high fat diets

<table>
<thead>
<tr>
<th>Calorie composition (% kcal)</th>
<th>Chow diet</th>
<th>High fat diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>69</td>
<td>21</td>
</tr>
<tr>
<td>Proteins</td>
<td>19</td>
<td>19</td>
</tr>
<tr>
<td>Total fats</td>
<td>11</td>
<td>60</td>
</tr>
<tr>
<td>Total energy from diet (kcal/g)</td>
<td>3.3</td>
<td>5.21</td>
</tr>
</tbody>
</table>
2.2.2. Longitudinal body weight and food intake analysis

To examine energy intake the mice had food intake and body weight monitored weekly. As the animals were group housed, food intake for the whole cage was weighed and divided by the number of mice per cage to calculate the amount of food eaten per mouse each week. During this period any deaths were recorded for survival analysis. Caloric intake was calculated by multiplying the amount of diet eaten in g by the physiologic fuel value of the diet (3.3kcal/g for standard laboratory chow and 5.21 kcal/g for the HF diet).

2.2.3. Indirect calorimetric measurement of metabolic rate

To measure metabolic rate the mice were placed in comprehensive lab animal monitoring system (CLAMS) calorimetry cages (Columbus Instruments, Columbus, OH, USA) for 5 d (see Figure 2.1). The body weight of each mouse was entered into the system to allow calculation of metabolic rate, which takes body weight into account. Air samples were taken from each cage every 10 min. Moisture was initially removed from the sample by a column of desiccant before passage through a sensor, which measured the levels of oxygen consumption ($VO_2$) and carbon dioxide production ($VCO_2$) produced. After 24 h habituation, $VO_2$ and carbon $VCO_2$ were measured every 10 min for 4 d. The respiratory quotient (RQ) was then calculated by dividing $VCO_2$ produced by $VO_2$ consumed. During the period in the CLAMS calorimetry cages, animals were given nesting material and were provided with ad libitum access to food and water. Body weight, food intake and water intake were measured daily. Energy balance over the 4 d was calculated as the caloric intake (kcal/day) minus heat energy expenditure (heat kcal/day). Heat kcal/h was calculated directly by the CLAMS apparatus.

![Figure 2.1. Comprehensive Laboratory Animal Monitoring System (CLAMS)](image)

*To measure metabolic rate the mice were housed individually in comprehensive lab animal monitoring system (CLAMS) indirect calorimetry cages for 5 d. After 24 h habituation, oxygen consumption ($VO_2$) and carbon dioxide production ($VCO_2$) were measured every 10 min for 4 d.*
2.3. Assessment of behaviour and memory

To evaluate behaviour and memory, cohorts of male Non-Tg control and 3xTgAD mice were subjected to open-field (OF), Y-maze spontaneous alternation (SA), smell recognition (SR), novel object recognition (NOR) and Morris water maze (MWM) tasks. A separate cohort of C57BL/J6 and 129SV mice were also subjected to the OF and SA task to determine any potential strain specific differences in behaviour and memory. On the days of behavioural evaluation, home cages were placed in the testing room 30 min prior to testing to allow habituation. All behavioural observations were made between 10.00am and 4.00pm. The order of observation during this period was randomised across animals. No more than one behavioural task was completed during any single day. The OF, SA, SR/NOR arenas and mazes did not have bases, therefore blue roll (Kimberly-Clark, UK) was placed underneath and replaced between animals. Except for the MWM, all arenas, mazes and objects were cleaned with 70 % ethanol between animals.

2.3.1. Open-field (OF) behaviour test

The OF behaviour test is a measure of exploration, general activity levels and anxiety in response to a novel environment.

Apparatus

The OF arena consisted of a red opaque Perspex box placed on the floor of a behavioural testing room (see Figure 2.2.). A video camera (Vista protos IV, UK) held 1 m above the arena by a tripod was connected to a computer with 2020 PLUS tracking software (HVS Image, Buckingham, UK). The seated experimenter was situated approximately 3 m away from the OF arena, out of sight of the animals. A live video feed of the observation was visible to the experimenter on an Acer laptop connected to the video camera, allowing the experimenter to score the observation.

Behavioural procedure

Each animal was placed in the centre of the OF arena (see Figure 2.2.) and allowed to explore for 5 min.
The experimenter observed the live video feed and noted the total number of times the animal reared within the arena. Rearing was defined as the behaviour where a mouse goes on its hind legs. It is used as a measure of environmental exploration. The total number included rearing in the centre of the arena as well as against the arena walls. The total number of defecations in the arena was also recorded. This can be a measure of anxiety, as a more anxious mouse will defecate more in a novel environment (Gimenez-Llort et al. 2007).

**OF test analysis**

The 2020 PLUS tracking software (HVS Image, Buckingham, UK) divided the arena into 16 cells and calculated the number of cell entries and the percentage of time spent moving. The number of rearings and defecations were totalled for each animal.

**2.3.2. Y-maze continuous spontaneous alternation (SA) test**

The SA test is a measure of short-term working memory and also a measure of exploratory activity. As the behaviour in this task is not reinforced by a reward or foot shock it is considered “spontaneous”.

**Apparatus**

A black opaque Perspex Y-maze (see Figure 2.4.) with arms labelled A, B and C was used for the SA test. The maze contained cues located at the end of each arm to assist the mouse in identifying
Chapter 2

General materials and methods

Each arm (see Figure 2.3. for arm cues). Cues were also available around the room to further assist the mouse. The maze was placed on a table with the seated experimenter located directly in front of arm A. Thus the experimenter also acted as a spatial cue.

![Diagram of a Y-maze with arm cues]

**Figure 2.3. Intra-maze cues in the Y-maze spontaneous alternation (SA) test**

Intra-maze cues (A, B and C) located at the end of arms in the Y-maze SA test.

**Behavioural procedure**

Each animal was placed in arm A of the Y-maze (see Figure 2.4.) and allowed to explore for 8 min. During this period the arm entries made by each animal were recorded manually by the seated experimenter.

![Diagram of a Y-maze with bird and measurements]

**Figure 2.4. Y-maze spontaneous alternation (SA) test**

In turn, each animal was then placed in arm A of the Y-maze and allowed to explore for 8 min. During this period the total number and specific arm choices were recorded.
SA test analysis

Continuous SA was defined as a successive entry into three different arms, on overlapping triplet sets. The best strategy to escape the maze is to check each arm in succession to determine if there is an exit. The SA test is thought to be a test of working memory as the mouse must use the cues located at the end of each arm and around the experimental behaviour room to determine which arm they were not in previously (Hughes 2004). Examining the total number or arm choices is also a measure of exploratory behaviour. The percentage number of alternations was calculated as the number of actual alternations divided by the maximum number of alternations (the total number of arm entries minus two).

2.3.3. Odour recognition (OR) test

The odour recognition (OR) test is a test of short-term non-associative memory based on the natural exploration of novelty in mice (Saddoris 2006; 2007).

Apparatus

The OR arena consisted of a black opaque polycarbonate circular arena (see Figure 2.6.) with no base. Scented balls were placed in the centre of the OR arena 5 cm from the edge of the arena and 8 cm away from each other. The scented balls consisted of a yellow hollow ball (6 cm diameter, Chad Valley, UK) which had small holes punched into them (0.3 cm diameter). Into these holes cotton wool was inserted until the ball was completely full. The morning of the behavioural testing the balls were wiped clean with 70% ethanol and then a total of 0.5 ml of scent (orange, lemon, vanilla or almond, Dr. Oetker Ltd, UK) was evenly distributed across all the holes onto the cotton wool. This procedure was performed in another room 30 min before behavioural testing while the animals habituated to the behavioural testing room. Once scented, the balls were individually kept in airtight zip lock bags until required. The balls were returned to their bags when not in use. A camera (Sanyo Xacti VPC-C4, SANYO Fisher, CA) held 1 m above the OR arena recorded colour MP4 video clips of each animal performing the task.

Behavioural procedure

Habituation

Animals from each cohort were placed in turn in an empty OR arena and allowed to explore for 5 min. Cohorts were habituated over 2 d in a randomised order throughout the day.
Trials

After habituation all cohorts underwent the OR test, which comprised of two phases (see Figure 2.5. for time-line).

During phase 1, the mouse was placed in the OR arena and allowed to explore two identical unfamiliar scented balls for 10 min (see Figure 2.6). The mouse was then housed individually in a clean new holding cage with *ad libitum* access to food and water for 3 min. During this time one of the two balls the mouse was previously allowed to explore was removed and replaced with a novel scented ball (see Table 2.2. for scent combinations). The novel scented ball was randomly placed in either the left or right position of one of the previously explored balls. During the second phase, the mouse was placed back into the arena and allowed to explore the familiar scented ball and the novel scented ball for 4 min (see Figure 2.6).

**Table 2.2. Scent combinations of the odour recognition (OR) test**

*During phase 1, the mouse was presented with two identical scents of vanilla (V), orange (O), almond (A) or lemon (L). During phase 2, the mouse had either the left or the right familiar scent replaced with a novel scent according to the table. For example, if the mouse was presented with two vanilla scented balls during the phase one they were presented with either orange or lemon replacing the vanilla on the left or the right side of the OR arena.*

<table>
<thead>
<tr>
<th>Phase 1</th>
<th>Phase 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>V V</td>
<td>V O, O V, V L, L V</td>
</tr>
<tr>
<td>O O</td>
<td>O A, A O, O V, V O</td>
</tr>
<tr>
<td>A A</td>
<td>A O, O A, A L, L A</td>
</tr>
<tr>
<td>L L</td>
<td>L V, V L, L A, A L</td>
</tr>
</tbody>
</table>
Chapter 2  
General materials and methods

**OR test analysis**

MP4 video-clips recorded during OR trials were converted to an AVI format using Pazera MP4 to AVI converter 1.3 (Pazera-Software, PL). The duration (seconds) spent exploring the scented balls was then measured using Observer 5.0 software (Noldus, Wageningen, The Netherlands). Exploration was defined as the amount of time the animals spent with their nose pointing within 2 cm of the scented balls. The percentage time spent exploring the scented balls was calculated for phase 1 and phase 2.

---

**Figure 2.6. Schematic representation of the odour recognition (OR) test.**

The OR test consisted of two phases 1 (A) and 2 (B). During phase 1, the mouse was placed in the OR arena facing the wall and allowed to explore the arena and two identically-scented balls for 10 min. After an interval of 3 min (phase 2) one of the familiar scented balls was substituted by a novel scented ball (in the identical position) and the mouse was again allowed to explore the arena for 4 min. The amount of time spent exploring the novel scented ball in comparison to the familiar scented ball (measured as the amount of time the nose spent within 2 cm of the object) indicated the ability of the mouse to determine whether the scent of the ball was novel or not.
2.3.4. Novel object recognition (NOR) test

The NOR test is a test of short-term non-associative memory based on the natural exploration of novelty in mice (Ennaceur 2010; Ennaceur & Delacour 1988; Ennaceur & Meliani 1992).

Apparatus

The NOR arena consisted of a black opaque polycarbonate circular arena (30 cm) with no base (see Figure 2.8.) Two wooden painted trial objects, varied in shape and colour (Universe of Imagination, Geoffrey, Inc., U.K.), were placed in the centre of the NOR arena, 5 cm from the edge of the arena and 8 cm away from each other. A camera (Sanyo Xacti VPC-C4, SANYO Fisher, CA) held 1 m above the NOR arena recorded colour MP4 video clips of each animal performing the task.

Behavioural procedure

Habituation

Animals from each cohort were placed in turn in an empty NOR arena and allowed to explore for 5 min. Cohorts were habituated over 2 d in a randomised order throughout the day.

Trials

After habituation all cohorts underwent the NOR test which comprised of two trial phases (see Figure 2.7. for time-line).

During trial phase 1, the mouse was placed in the NOR arena and allowed to explore two identical unfamiliar objects for 10 min (see Figure 2.8.). The mouse was then housed individually in a clean new holding cage with ad libitum access to food and water for an interval of 1 h. During this time, one of the two objects the mouse was previously allowed to explore was removed and replaced.
with a novel object. During trial phase 2, the mouse was placed back into the arena and allowed to explore the familiar object and the novel object for 4 min (see Figure 2.8.).

A. Trial phase 1

2 identical novel objects

B. Trial phase 2

1 familiar and 1 novel object

Figure 2.8. Schematic representation of the Novel Object Recognition (NOR) test

Mice were individually habituated to the arena for 5 min for 2 d prior to the NOR test. The NOR test consisted of two trial phases 1 (A) and 2 (B). During trial phase 1 the mouse was placed in the NOR arena facing the wall and allowed to explore the arena and two identical unfamiliar objects for 10 min. After an interval of 1 h (trial phase 2) one of the familiar objects was substituted by a novel object (in the identical position) and the mouse was again allowed to explore the arena for 4 min. The amount of time spent exploring the novel object in comparison to the familiar object (measured as the amount of time the nose spent within 2 cm of the object) indicated the ability of the mouse to determine whether an object was novel or not.

NOR test analysis

MP4 video clips recorded during NOR trials were converted to an AVI format using Pazera MP4 to AVI converter 1.3 (Pazera-Software, PL). The duration (seconds) spent exploring the objects was then measured using Observer 5.0 software (Noldus, Wageningen, The Netherlands). Object exploration was defined as the amount of time animals spent with their nose pointing within 2 cm of an object. The percentage of time spent exploring objects was calculated for phase 1 and phase 2.
2.3.5. Morris water maze (MWM) task

The MWM is a test of spatial reference memory (Morris et al 1982).

**Apparatus**

**MWM**

The MWM consisted of a circular white opaque plastic tank 1.2 m in diameter with walls 25 cm high (see Figure 2.9.).

**MWM room configuration and experimenter position**

The MWM was located in the centre of a room, surrounded by interlocking screens to mask the surrounding environment (see Figure 2.9.). This was done to keep the conditions around the maze consistent between trials and experiments, as well as to hide the position of the experimenter and stop the experimenter from becoming a distal cue. Once the mouse was placed in the water the experimenter hid from view and remained in position next to the laptop where the trial could be observed. A CCTV tracking camera (Vista protos IV, UK) with Water 2010 software from HVS image was over the MWM.

**MWM platform**

The platform consisted of a square piece of acrylic plastic (10 cm by 10 cm) with an acrylic dowel as the vertical post, which was attached to a square piece of metal.

**MWM flagged cue**

The flagged cue consisted of a wooden cylinder painted red (6 cm high by 3 cm diameter). The top of the object was covered in white masking tape to avoid detection from the tracking system, when placed on top of the platform during the flagged platform-learning phase.
Figure 2.9. Set-up of the Morris water maze (MWM)

The MWM consisted of a circular white opaque plastic tank 1.2 m in diameter with walls 25 cm high. The water was made opaque using water-soluble white paint and maintained at a temperature of 21-22 °C. The platform consisted of a square piece of acrylic plastic with an acrylic dowel as the vertical post, which was attached to a square piece of metal. During flagged and submerged platform training, the platform was 0.5 cm under the water level, making it invisible to the mouse. The MWM was located in the centre of a room, surrounded by interlocking screens to mask the surrounding environment. This was done to keep the conditions around the maze consistent between trials and experiments, to hide the position of the experimenter and to stop the experimenter from becoming a distal cue. Above the water maze, a CCTV camera was located to track the mice during swimming. The camera was positioned between two strip lights, centrally located above the pool. The camera was connected to a laptop with Water 2010 tracking software by HVS image. During flagged platform training, no extra maze spatial cues were present, other than a red wooden block placed on top of the platform to guide the mouse towards the platform. During submerged platform training and the probe trial, extra maze spatial cues were placed on the inside of the screen to aid spatial navigation.
Extra maze spatial cues

During submerged platform training and the probe trial, spatial cues were placed on the inside of the screen to aid spatial navigation. These consisted of four 3-dimensional objects (see Figure 2.10. for objects and Figure 2.9. for object locations) differing in size, colour and texture.

![Figure 2.10. Extra maze spatial cues in the Morris water maze (MWM)](image)

Extra maze spatial cues located around the MWM used to locate the platform during submerged platform training and the probe trial.

Water

The water was made opaque using water-soluble white paint (Universe of Imagination, Geoffrey Inc., U.K.) and maintained at a temperature of 21-22 °C. Prior to each day of training, the water temperature was measured. Water was removed each day until the platform was visible. Hot white opaque water was then used, to replace the water removed, until the desired water temperature was obtained and reached the desired water level 0.5 cm above the platform, so that the platform was no longer visible. The paint at the bottom of the water maze was also stirred each day.
Tracking system

Above the water maze, a CCTV camera was located to track the mice during swimming. The camera was positioned between two strip lights, centrally located above the pool and was connected to a laptop that contained Water 2010 tracking software by HVS image (see Figure 2.9.). The water maze tracking software was calibrated and configured prior to the water maze training. The aperture of the camera was adjusted to minimise reflectance of water up to the camera, which may interfere with tracking. The border locations of the pool were then defined, including placement of platform locations. The tracking software was then tested to make sure the mouse was being tracked correctly.

Behavioural procedure

The MWM task consisted of flagged platform training, submerged platform training and a probe trial (see Figure 2.11 for a time-line) based on protocol by Vorhees & Williams (2006).

Flagged platform training

As a control, prior to MWM submerged platform training, a flagged learning task was performed. This pre-training involved the mouse swimming from a series of random start positions to a set of random flagged platform locations (see Table 2.3.). Mice were either given one (see chapter 4) or two days (see chapter 5) of flagged platform training. No extra maze spatial cues were present during this stage of the task, therefore making it a non-spatial task. This experimental pre-training procedure teaches the mice that only by searching for the proximal cue located on the submerged platform can they escape. This non-spatial pre-training teaches the mice the basic sensorimotor requirements and procedure of the MWM task (Vorhees & Williams 2006). The mouse was placed in the desired start position of the MWM at water level, facing the tank wall. As soon as the mouse was released, the tracking program was started. Once the mouse reached the platform, the timer...
was stopped. If the mouse did not reach the platform within the trial limit of 2 min, they were guided to it. Once on the platform, the mouse was allowed to remain there for 10 s to further orient itself. If the mouse jumped off the platform, they were guided back to it and held in position on the platform for 10 s. After this time, the mouse was dried and placed in a holding cage until all the other mice in that cage performed the first trial. Once all the mice performed trial one, then the process started again with the next start position and flagged platform location for trial two (see Table 2.3. for MWM flagged platform start positions). The procedure was repeated until all the mice performed 4 trials that day (Vorhees & Williams 2006). Mice were given four trials a day for 1 (see chapter 4) or 2 d (see chapter 5).

**Table 2.3. Flagged platform training trial pattern of the Morris water maze (MWM) test**

The first letter denotes the start position (N; north, S; south, E; east, W; west) and the second letter denotes the cued platform position (NE; north east, NW; north west, SE; south east, SW; south west). Table taken from MWM protocol by Vorhees & William (2006).

<table>
<thead>
<tr>
<th>Test day</th>
<th>Trial 1</th>
<th>Trial 3</th>
<th>Trial 3</th>
<th>Trial 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>N-SE</td>
<td>E-NE</td>
<td>S-SW</td>
<td>W-SE</td>
</tr>
<tr>
<td>2</td>
<td>S-NE</td>
<td>N-NW</td>
<td>W-NE</td>
<td>E-SE</td>
</tr>
</tbody>
</table>

**Submerged platform training**

To test spatial reference, memory mice underwent submerged platform training in the MWM. Trials were performed as during the flagged platform training with the following differences; spatial cues were put in place (see Figure 2.10. for cues and Figure 2.9 for cue locations) to aid navigation during submerged platform training and no flag was placed on top of the platform. The platform was placed in the SW position of the MWM (see Figure 2.9.) where it remained for all the trials. Mice were given four trials a day for 5 (see chapter 4) or 8 d (see chapter 5) (see Table 2.4. for submerged platform start positions). Mice were given four different start positions each day and were required to use the spatial cues, situated around the MWM, to locate the platform. Decreasing escape latency over successive training days indicated learning (Vorhees & Williams 2006).
To test memory retention of the platform location, mice underwent a probe trial 24 h after the final submerged platform training trial. During the probe trial, the platform was removed from the MWM. The mouse was then placed in a novel start position in the MWM (see Table 2.4.) and allowed to swim for a fixed interval of 30 s. After this time, the mouse was then removed, dried and returned to its home cage. A mouse that has remembered the location of the platform should spend most of their time swimming around where the platform was previously located (Vorhees & Williams 2006).

**Probe trial**

To test memory retention of the platform location, mice underwent a probe trial 24 h after the final submerged platform training trial. During the probe trial, the platform was removed from the MWM. The mouse was then placed in a novel start position in the MWM (see Table 2.4.) and allowed to swim for a fixed interval of 30 s. After this time, the mouse was then removed, dried and returned to its home cage. A mouse that has remembered the location of the platform should spend most of their time swimming around where the platform was previously located (Vorhees & Williams 2006).

**MWM task analysis**

2020 PLUS tracking software (HVS Image, Buckingham, UK) calculated the escape latency and swim speed, during both flagged platform and submerged platform training for each mouse over trials, and the percentage time in the target quadrant, during the probe trial for each mouse. Mean escape latency and swim speed over four trials within a day was calculated for each mouse.

**Table 2.4. Morris water maze (MWM) submerged platform training and probe trial start position pattern**

The platform was located in the south west (SW) quadrant of the MWM during submerged platform training. Each testing day of submerged platform training included one trial from each of the four start positions (N; north, NW; north west, E; east, SE; south east. A probe test was performed 24 h after the final trial of submerged platform training. The platform was removed and the mouse was placed in the north east (NE) quadrant of the MWM. Table taken from MWM protocol by Vorhees & Williams (2006).

<table>
<thead>
<tr>
<th>Test day</th>
<th>Trial 1</th>
<th>Trial 2</th>
<th>Trial 3</th>
<th>Trial 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>N</td>
<td>E</td>
<td>SE</td>
<td>NW</td>
</tr>
<tr>
<td>2</td>
<td>SE</td>
<td>N</td>
<td>NW</td>
<td>E</td>
</tr>
<tr>
<td>3</td>
<td>NW</td>
<td>SE</td>
<td>E</td>
<td>N</td>
</tr>
<tr>
<td>4</td>
<td>E</td>
<td>NW</td>
<td>N</td>
<td>SE</td>
</tr>
<tr>
<td>5</td>
<td>N</td>
<td>SE</td>
<td>E</td>
<td>NW</td>
</tr>
<tr>
<td>6</td>
<td>SE</td>
<td>N</td>
<td>NW</td>
<td>E</td>
</tr>
<tr>
<td>7</td>
<td>E</td>
<td>NW</td>
<td>SE</td>
<td>N</td>
</tr>
<tr>
<td>8</td>
<td>NW</td>
<td>E</td>
<td>N</td>
<td>SE</td>
</tr>
<tr>
<td>9 (probe)</td>
<td>NE</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
2.4. Culling

2.4.1. Perfusion

Prior to culling, the animals had their body weight recorded. The animals were then terminally anaesthetised with 3.5 % isoflurane (30 % O₂ and 70 % N₂O) and the chest cavity opened, to expose the heart by cutting through the diaphragm and rib cage. A butterfly needle was then inserted into the same puncture site in the left ventricle used to take the blood sample and clamped. An incision was then made in the right atrium, after which 0.9 % saline (treated with 1ml Diethylpyrocarbonate (DEPC) overnight, autoclaved then stored at 4°C) was perfused transcardially at a rate of 20 ml/min, using a perfusion pump (Harvard Apparatus, UK) for 2 min.

2.4.2. Tissue extraction

After saline perfusion, all tissue samples were removed and immediately placed on dry ice to prevent degradation. Tail snips were taken and genotyped (see Appendix B). Epididymal fat surrounding the testicles of the male mice were removed and weighed before freezing. Liver, spleen and kidney were also taken and stored at -80°C for future analysis. The brain was rapidly removed and cut sagittally with a razor blade. The cerebellum, hippocampus and cortex were excised from the right hemisphere of the brain for future biochemical analysis. The pituitary was also taken. The left hemisphere was post-fixed in 5 ml of 4% paraformaldehyde (PFA) at room temperature for 24 h. The brain was then cryoprotected in 30% sucrose (in 0.1 M phosphate buffer (PB)) at 4°C. Once sunk (approximately 24 h), the brain was carefully removed, snap-frozen in isopentane on dry ice and stored at -80°C until future sectioning for histological analysis.

2.5. Statistical analysis

Data are represented as mean ± standard error of the mean (SEM). See individual chapters for detailed statistical analysis. Statistical significance levels are as follows: *p < 0.05, **p < 0.01 and ***p < 0.001.
Chapter 3

Characterisation of energy balance in 3xTgAD mice
3.1. Introduction and objectives

Energy balance appears to be affected throughout the progression of AD. While mid-life obesity increases the risk of developing AD (Fitzpatrick et al. 2009; Rosengren et al. 2005; Whitmer et al. 2005; Whitmer et al. 2008), life-threatening weight loss occurs and worsens as the disease progresses (Gillette-Guyonnet et al. 2007; Gillette-Guyonnet et al. 2000; Guerin et al. 2005; White et al. 1996). The preliminary aim of this PhD, therefore, was to examine potential differences in energy balance in the 3xTgAD mouse model of AD, compared to Non-Tg control mice. In order to evaluate energy balance, body weight, food intake and metabolic rate were examined in cohorts of 3xTgAD and Non-Tg control mice at 2, 12 and 18 months of age. A separate cohort of 129SV and C57BL/J6, the individual background strains upon which 3xTgAD mice were created, were also monitored to examine potential strain specific differences in body weight, food intake and metabolic rate.

3.2. Materials and methods

3.2.1. Experimental design

Cohorts of 3xTgAD and age-matched Non-Tg control mice as well as 129SV and C57BL/J6 mice had energy balance monitored (see Table 3.1.). Cohorts 1, 2 and 4 had weekly body weight and food intake measured from weaning until 2, 12 or 20 months of age (see Method 2.2.1. - 2.2.2). At the end of the monitoring mice from each cohort were placed into CLAMS calorimetry cages to evaluate metabolic rate (see Method 2.2.3). Over this period, body weight, food intake and water intake were monitored (see Method 2.2.3.) and a 24 h average calculated. The mice were then culled (see Method 2.4.).

Table 3.1. Experimental cohorts for energy balance assessment (comprehensive laboratory animal monitoring system; CLAMS)

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Monitoring period</th>
<th>Gender</th>
<th>Genotype</th>
<th>Cohort size</th>
<th>Cohort in CLAMS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4 weeks - 2 months</td>
<td>Male</td>
<td>Non-Tg 3xTgAD</td>
<td>n = 8</td>
<td>n = 4</td>
</tr>
<tr>
<td>2</td>
<td>4 weeks - 12 months</td>
<td>Male</td>
<td>Non-Tg 3xTgAD</td>
<td>n = 6</td>
<td>n = 10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Female</td>
<td>Non-Tg 3xTgAD</td>
<td>n = 9</td>
<td>n = 4</td>
</tr>
<tr>
<td>3</td>
<td>18 months</td>
<td>Male</td>
<td>Non-Tg 3xTgAD</td>
<td>n = 4</td>
<td>n = 4</td>
</tr>
<tr>
<td>4</td>
<td>4 weeks - 20 months</td>
<td>Male</td>
<td>129SV C57BL/J6</td>
<td>n = 6</td>
<td>n = 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
3.2.2. Statistical analysis

Longitudinal body weight between 3xTgAD and Non-Tg control mice and between 129SV and C57BL/J6 was examined using repeated measures analysis of variance (ANOVA) with SigmaStat 4 (Systat Software Inc., IL, USA). Differences in survival were determined using Logtest survivalship curve analysis, with Prism (Graphpad, San Diego, CA, USA). No statistical analysis was performed on estimated average weekly food intake per mouse, as these data were derived from group-housed animals. Statistical differences between 3xTgAD and Non-Tg cohorts and between 129SV and C57BL/J6 mice for 24 h average body weight, food intake, water intake, volume of oxygen consumption (VO₂), volume of carbon dioxide production (VCO₂) and respiratory quotient (RQ) over the 4 d in CLAMS calorimetry cages were determined using Student’s t-tests with Prism (Graphpad, San Diego, CA, USA).
3.3. Results

3.3.1. Characterisation of the energy balance profile in 3xTgAD mice

3.3.1.1. 2-month-old male 3xTgAD mice displayed increased body weight and food intake

Between 6 and 8 weeks of age, 3xTgAD mice displayed 27 - 31% higher body weight ($p < 0.05$) compared with Non-Tg control mice (Figure 3.1.A). Estimated average weekly food intake per mouse, between 5 - 8 weeks of age, appeared greater in 3xTgAD (18 - 55%) than Non-Tg control mice (Figure 3.1.B).

**Figure 3.1.** 2-month-old male 3xTgAD mice displayed increased body weight and food intake

Weekly body weight (A) and food intake (B) were monitored in cohorts of male 3xTgAD (n = 8) and Non-Tg control mice (n = 8) from weaning to 2 months of age. Body weight is represented as mean ± SEM. Repeated measures analysis of variance (ANOVA), *$p < 0.05$. 

53
3.3.1.2. 12- month -old 3xTgAD displayed altered body weight and food intake profiles

Over a 12-month period, male 3xTgAD mice had different body weight (Figure 3.2.A) and food intake (Figure 3.2.B) profiles, compared to Non-Tg control mice. Between 5 weeks and 4 months of age 3xTgAD mice displayed 12 - 33% higher ($p < 0.05$) body weight than Non-Tg control mice. In contrast, between 7.5 - 8.5 and 9.5 - 12 months of age, body weight was lower (by 8 - 9% and 11 - 18% respectively) in 3xTgAD compared to Non-Tg control mice ($p < 0.05$). However, despite these biphasic differences in body weight in 3xTgAD mice, the estimated average weekly food intake per mouse was greater throughout the monitoring period, as over the 12 months, 3xTgAD mice ate 37% more food than Non-Tg controls.

Over 12 months, female 3xTgAD mice displayed no significant difference in body weight (Figure 3.2.C) compared to female Non-Tg control mice. The estimated average weekly food intake per mouse was greater throughout the monitoring period, and over the 12 months, female 3xTgAD mice ate 16% more food (Figure 3.2.D). A marked variation in body weight and food intake was observed between animals in the female cohort, becoming more apparent with increasing age.

**Figure 3.2. 3xTgAD mice had altered body weight and food intake profiles**

Cohorts of male and female 3xTgAD ($n = 6$ and $n = 8$ respectively) and Non-Tg control mice ($n = 6$ and $n = 9$ respectively) had weekly body weight (A & C) and food intake (B & D) measured over 12 months. Body weight is represented as mean ± SEM. Repeated measures analysis of variance (ANOVA), *$p < 0.05$.**
3.3.1.3. Decreased survival in female 3xTgAD mice

At the end of the 12-month body weight and food intake monitoring period, survival was examined in male (Figure 3.3.A) and female (Figure 3.3.B) 3xTgAD and Non-Tg controls. At 12 months of age, there was a 100% survival rate for both male and female Non-Tg control mice (n = 6 and n = 9 respectively). No significant difference in survival curves was observed between male 3xTgAD (n = 6) and Non-Tg (n = 6) control mice (p > 0.05), as only one death was observed in the male 3xTgAD cohort at 9 months of age. In contrast, there was a decrease (p < 0.01) in survival rate in female 3xTgAD, when compared to Non-Tg controls. Between 6-12 months of age, five deaths were observed in a cohort of eight female 3xTgAD mice. By 12 months of age there was 37% survival within the female cohort. Due to decreased survival observed in female 3xTgAD mice, subsequent studies were only performed in male mice.

**Figure 3.3. Decreased survival in female 3xTgAD mice**

Over 12 months, survival was examined in cohorts of male (A) and female (B) 3xTgAD (male, n = 6 and female, n = 8) and Non-Tg control mice (male, n = 6 and female, n = 9). Logtest survivalship curve test, **p < 0.01.**
3.3.1.4. 2-month-old 3xTgAD mice displayed no difference in metabolic rate

When housed individually in CLAMS calorimetry cages at 2 months of age, a greater mean body weight (34%; \( p < 0.001 \), Figure 3.4.A), 24 h food intake (17%; \( p < 0.05 \), Figure 3.4.B) and water intake (42%; \( p < 0.05 \) Figure 3.4.C) was observed in 3xTgAD mice, when compared to Non-Tg control mice. Indirect calorimetry at 2 months of age, to measure metabolic rate, revealed that both 3xTgAD and Non-Tg control mice display similar 24 h rhythms in VO\(_2\) consumption (Figure 3.4.D), VCO\(_2\) production (Figure 3.4.E.) and RQ (Figure 3.4.F). VO\(_2\), VCO\(_2\) and RQ levels were lowest during the light, largely inactive, phase of the day and highest during the dark, active phase. Both VO\(_2\) and VCO\(_2\) levels peaked early during the dark phase. No statistical differences in mean 24 h VO\(_2\) (Figure 3.4.G.), VCO\(_2\) (Figure 3.4.H.) or RQ (Figure 3.4.I.) were detected between 3xTgAD and Non-Tg control mice. During the monitoring period, both groups of mice exhibited a similar degree of positive energy balance (Non-Tg, 5.2 ± 0.3 kcal/day versus 3xTgAD, 5.4 ± 0.7 kcal/day).

![Figure 3.4. 2-month-old 3xTgAD mice displayed no difference in metabolic rate](image URL)

At 2 months of age, cohorts of male 3xTgAD (n = 4) and Non-Tg (n = 4) control mice were individually housed in Comprehensive Lab Animal Monitoring System (CLAMS) calorimetry cages for four days to evaluate metabolic rate. During the period in CLAMS, 3xTgAD mice had mean 24 h body weight (A), food intake (B) and water intake (C) monitored. Rhythms in VO\(_2\) (D), VCO\(_2\) (E) and RQ (F) and mean 24 h VO\(_2\) (G), VCO\(_2\) (H) and RQ (I) were assessed. Data are mean ± SEM. Student’s t-tests, \(* p < 0.05, \*\* p < 0.001\). (D, E, F), white bars represent the light, inactive phase of the day whereas black bars represent the dark active phase.
3.3.1.5. 12-month-old 3xTgAD mice had a higher metabolic rate

When housed individually in calorimetry cages, 12-month-old 3xTgAD mice exhibited a lower mean 24 h body weight (15%; \( p < 0.01 \), Figure 3.5.A), despite a 30% higher mean 24 h food intake (\( p < 0.05 \), Figure 3.5.B), compared to Non-Tg control mice. No significant difference in water intake (Figure 3.5.C) was observed between 3xTgAD and Non-Tg control mice. At 12 months of age indirect calorimetry, to measure metabolic rate, revealed that both 3xTgAD and Non-Tg control mice display 24 h rhythms in \( \text{VO}_2 \) consumption (Figure 3.5.D), \( \text{VCO}_2 \) production (Figure 3.5.E) and RQ (Figure 3.5.F). \( \text{VO}_2 \), \( \text{VCO}_2 \) and RQ levels were lowest during the light, largely inactive, phase of the day and highest during the dark, active phase. Both \( \text{VO}_2 \) and \( \text{VCO}_2 \) levels peaked early during the dark phase. During the light and dark phase, \( \text{VO}_2 \) and \( \text{VCO}_2 \) rhythms were higher in 3xTgAD, compared to Non-Tg control mice. Rhythms in RQ were visible, but more variable in both 3xTgAD and Non-Tg mice. RQ peaked early during the dark phase in 3xTgAD mice and later in the dark phase in Non-Tg control mice. Statistical analysis revealed that 3xTgAD mice had a higher metabolic rate, as assessed by a greater 24 h mean \( \text{VO}_2 \) (24%; \( p < 0.01 \), Figure 3.5.G) and \( \text{VCO}_2 \) (29%; \( p < 0.001 \), Figure 3.5.H), when compared with Non-Tg control mice. No significant difference in mean daily RQ was observed between 3xTgAD and Non-Tg control mice (Figure 3.5.I). During the monitoring period, both groups of mice exhibited a positive energy balance and there was no significant difference between groups (Non-Tg, 4.3 ± 1.9 kcal/day versus 3xTgAD, 5.8 ± 0.8 kcal/day).
At 12 months of age, cohorts of male 3xTgAD (n = 5) and Non-Tg (n = 6) control mice were individually housed in Comprehensive Lab Animal Monitoring System (CLAMS) calorimetry cages for four days to evaluate metabolic rate. During the period in CLAMS, 3xTgAD mice had mean 24 h body weight (A), food intake (B) and water intake (C) monitored. Rhythms in VO$_2$ (D), VCO$_2$ (E) and RQ (F) and mean 24 h VO$_2$ (G), VCO$_2$ (H) and RQ (I) were assessed. Data are mean ± SEM. Student’s t-tests, *p < 0.05, **p < 0.01, ***p < 0.001. (D, E, F), white bars represent the light, inactive phase of the day whereas black bars represent the dark, active phase.
3.3.1.6. 18-month-old 3xTgAD mice had a higher metabolic rate

When housed individually in calorimetry cages, 18-month-old 3xTgAD mice exhibited a lower mean 24 h body weight (13%; \( p < 0.01 \), Figure 3.6.A), despite a 14% higher mean 24 h food (\( p < 0.05 \), Figure 3.6.B) and water intake (69%; \( p < 0.01 \), Figure 3.6.C), compared to Non-Tg control mice. At 18 months of age, indirect calorimetry, to measure metabolic rate, revealed that 3xTgAD mice display 24 h rhythms in \( \text{VO}_2 \) consumption (Figure 3.6.D), \( \text{VCO}_2 \) production (Figure 3.6.E) and RQ (Figure 3.6.F). \( \text{VO}_2 \), \( \text{VCO}_2 \) and RQ levels in 3xTgAD mice were lowest during the light, largely inactive phase of the day and highest during the dark, active phase. For 3xTgAD mice, \( \text{VO}_2 \), \( \text{VCO}_2 \) and RQ peaked during the middle of the dark phase. At 18 months of age, Non-Tg mice displayed very weak rhythms in \( \text{VO}_2 \) and \( \text{VCO}_2 \). Rhythms in RQ were visible in Non-Tg mice, peaking around the onset of the dark phase. Rhythms in \( \text{VO}_2 \) and \( \text{VCO}_2 \) appeared higher in 3xTgAD mice compared to Non-Tg control mice during the light and dark phase, however the difference was greater during the dark phase. Statistical analysis revealed that 3xTgAD mice had a higher metabolic rate, as assessed by a greater 24 h mean \( \text{VO}_2 \) (23%; \( p < 0.01 \), Figure 3.6.G) and \( \text{VCO}_2 \) (24%; \( p < 0.01 \), Figure 3.6.H), when compared with Non-Tg control mice. No significant difference in mean daily RQ was observed between mice (Figure 3.6.I). During the metabolic monitoring period, both groups of mice exhibited a positive energy balance and there was no significant difference between groups (Non Tg, 5.14 ± 0.3 kcal/day versus 3xTgAD, 7.33 ± 1.1 kcal/day).
At 18 months of age, cohorts of male 3xTgAD (n = 4) and Non-Tg (n = 4) control mice were individually housed in Comprehensive Lab Animal Monitoring System (CLAMS) calorimetry cages for four days to evaluate metabolic data. During the period in CLAMS, 3xTgAD mice had mean 24 h body weight (A), food intake (B) and water intake (C) monitored. Rhythms in VO$_2$ (D), VCO$_2$ (E) and RQ (F) and mean 24 h VO$_2$ (G), VCO$_2$ (H) and RQ (I) were assessed. Data are mean ± SEM. Student’s t-tests, *p < 0.05, **p < 0.01. (D, E, F), white bars represent the light, inactive phase of the day whereas black bars represent the dark, active phase.
3.3.2. Characterisation of the energy balance profile in 129SV and C57BL/J6 strains

3.3.2.1. Male 129SV and C57BL/J6 mice displayed similar body weight and food intake profiles over 20 months

Over a 20-month period, there was no significant difference in growth profile (Figure 3.7.A) and food intake (Figure 3.7.B) between male 129SV and C57BL/J6 mice.

![Figure 3.7. Male 129SV and C57BL/J6 mice displayed similar body weight and food intake profiles over 20 months.](image)

Cohorts of male C57BL/J6 (n = 6) and 129SV (n = 6) mice had body weight (A) and food intake (B) monitored from weaning to 20 months of age. Body weight is represented as mean ± SEM. Repeated measures analysis of variance (ANOVA).
3.3.2.2. 20-month-old male 129SV and C57BL/J6 mice displayed similar body weight, food intake, water intake and metabolic rate

When individually housed in CLAMS calorimetry cages at 20 months of age, 129SV and C57BL/J6 mice displayed no significant differences in mean 24 h body weight (Figure 3.8.A), food intake (Figure 3.8.B) or water intake (Figure 3.8.C). Indirect calorimetry, to measure metabolic rate, revealed that 20-month-old 129SV and C57BL/J6 mice displayed similar 24h rhythms in VO₂ consumption (Figure 3.8.D), VCO₂ production (Figure 3.8.E) and RQ (Figure 3.8.F). VO₂, VCO₂ and RQ levels were lowest during the light, largely inactive phase of the day and highest during the dark, active phase. Both VO₂ and VCO₂ levels peaked early during the dark phase. No statistical differences in mean 24 h VO₂ (Figure 3.8.G), VCO₂ (Figure 3.8.H) or RQ (Figure 3.8.I) were detected between 129SV and C57BL/J6 mice.

Figure 3.8. 20-month-old male 129SV and C57BL/J6 mice displayed similar body weight, food intake, water intake and metabolic rate

At 20 months of age, cohorts of male 129SV (n = 4) and C57BL/J6 (n = 4) mice were individually housed in Comprehensive Lab Animal Monitoring System (CLAMS) calorimetry cages for four days to evaluate metabolic rate. During the period in CLAMS, 3xTgAD mice had mean 24 h body weight (A), food intake (B) and water intake (C) monitored. Rhythms in VO₂ (D), VCO₂ (E) and RQ (F) and mean 24 h VO₂ (G), VCO₂ (H) and RQ (I) were assessed. Data are mean ± SEM. Student’s t-tests. (D, E, F), white bars represent the light, inactive phase of the day whereas black bars represent the dark, active phase.
3.4. Discussion

Energy balance appears to be affected throughout the progression of AD. While mid-life obesity increases the risk of developing AD, life-threatening weight loss occurs and worsens as the disease progresses. The results of this PhD show that female 3xTgAD mice displayed no difference in energy balance profile, whereas male 3xTgAD mice showed an altered energy balance profile. We demonstrate for the first time that, at 2 months of age, 3xTgAD mice eat and weigh more than non-transgenic controls, while showing no changes in metabolism. In contrast, from 12 months of age, 3xTgAD mice continue to eat more than the controls, yet weigh less and display a hypermetabolic state. No difference in body weight, food intake and metabolic profiles was observed between 129SV and C57BL/J6 mice, therefore any alterations in energy balance observed in 3xTgAD mice is unlikely due to a background genetic effect.

3.4.1. Energy balance and survival in female 3xTgAD mice

Female 3xTgAD mice displayed no significant difference in body weight and food intake, when compared to age-matched Non-Tg control mice. This is the first time longitudinal analysis of body weight and food intake has been done in female 3xTgAD mice. Only one other study has reported body weight in female 3xTgAD mice, finding that they are significantly heavier at 12 months of age (Gimenez-Llort et al 2007). In the present study, there was variability in body weight in female mice, which increased with age. This increase in variability appeared to be linked with the reduced survival observed in female 3xTgAD mice after 6 months of age. This is the first time decreased survival has been noted in this model, though the reason for this is unknown. However, it has been seen in other transgenic models of AD, where 90% of male and 54.5% of female APP/PS1 mice survive to 10 months of age, whereas 100% of male and female wild-type controls survive (Pugh et al 2007). This is interesting, as it follows almost exactly what was observed in the present study. The authors discussed that prior to premature death, the mice did not show any changes in body weight or signs of illness and thus, the reason for premature death is unknown. Again, this is similar to what we observed, as death occurred very quickly. Reduced survival has also been noted in male and female Tg2576 mice through to 19 months of age (Arendash & King 2002; King & Arendash 2002), although no gender difference in survival was observed. The reasons for decreased survival in female 3xTgAD mice in the current study are currently unknown and require further examination.

3.4.2. Altered energy balance in male 3xTgAD mice

To our knowledge, this is the first time a longitudinal energy balance profile has been established from weaning in 3xTgAD mice. At 2 months of age, male 3xTgAD mice weighed significantly more than Non-Tg controls, but displayed no difference in metabolic rate. However, at 12 and 18 months
of age, male 3xTgAD mice weighed less and had a higher metabolic rate, compared with Non-Tg control mice. Male 3xTgAD mice were hyperphagic at all ages examined. No differences in body weight, food intake and metabolic rate were observed between individual background strains (129SV and C57BL/J6 mice) of 3xTgAD mice, therefore any alterations in energy balance observed in 3xTgAD mice is unlikely due to background genetic effect. In the present study we noted an increase in body weight early in life; however it is not known whether this increase was due to accelerated growth or an increased deposition of fat. No difference in body length was noted (data not shown), however more detailed analysis would be required in future studies, where body length and body composition (e.g. using DEXA) could be examined. Brain pathology was also assessed in the male cohorts in the present study (data not shown) (Knight et al 2010). At 2 months of age, intraneuronal Aβ pathology was seen in the cortex, hippocampus, and amygdala, and became more apparent by 12 months of age, when occasional Aβ plaques as well as a small number of hyperphosphorylated tau positive neurones were visible within the hippocampus. At 18 months of age, a more severe AD pathology was noted with increased Aβ plaques and hyperphosphorylated tau-positive neurones within the hippocampus. Thus, the shift towards a hypermetabolic state appears to start before significant disease pathology (plaques and tangles), although future studies would be required to determine exactly when changes in metabolic rate occur.

Very few studies have investigated body weight and food intake in 3xTgAD mice. One study reported that body weight of 2-month-old male 3xTgAD mice is either not different or increased depending on the individual experiment, while 6-month-old mice weigh more than controls (Gimenez-Llort et al 2007). At 6 months of age, we reported that 3xTgAD mice were no longer heavier than Non-Tg mice. Gimenez-Llort and colleagues examined body weight in separate groups of mice, at two time points, and did not continue after 6 months of age, making direct comparisons with the present study difficult. Another study did evaluate weekly body weight from 3 until 14 months of age, in a mixed male and female cohort (Halagappa et al 2007), however, details were not published, other than to say that at 14 months of age, 3xTgAD mice weigh on average 5-10 g less than Non-Tg mice. These data are similar to the present study where male 3xTgAD mice weighed approximately 5 g less than Non-Tg mice at 12 months of age. However, as we found gender differences in energy balance profiles, it is difficult to compare our data with those of Halagappa and colleagues. On the whole, in accordance with the present data, previous studies in 3xTgAD mice show that they are heavier earlier in life, and at some time point later, they are lighter than Non-Tg controls. No studies have previously examined longitudinal metabolic rate in any model of AD.
Chapter 3  Characterisation energy balance in 3xTgAD mice

3.4.3. Energy balance in other mouse models of AD

Currently very few studies have performed longitudinal studies, looking at body weight and food intake in other mouse models of AD. In contrast to the present data, the limited studies that have examined body weight in male and female APP and APP/PS-1 mice report that it is lower throughout life compared to control mice (Lalonde et al 2005; Lalonde et al 2002; Touma et al 2007). The majority of these studies, however, have only examined body weight at a single age and did not monitor food intake. Those studies that have looked at food intake found that both male and female APP 23 and APP/PS-1 mice eat more food, despite weighing less throughout their lives, suggesting altered energy expenditure, similar to that seen in the present study (Pugh et al 2007; Vloeberghs et al 2008). As discussed above, various APP and APP/PS-1 models of AD have lower body weight throughout life, which may be present from birth. However, the data presented in this PhD in 3xTgAD mice may mirror the onset and progression of AD more closely, as obesity is a known risk factor during mid-life, but life-threatening weight loss occurs as the disease progresses, despite adequate or increased food intake. These results, therefore, present for the first time, in a transgenic AD model, a biphasic body weight profile in the presence of increased food intake.

3.4.4. Why do aged male 3xTgAD mice have lower body weight?

In order to lose body weight, the energy gained from food (energy intake) must be less than the energy used (energy expenditure) resulting in a negative energy balance. This can be caused by decreased food intake and/or an increase in energy expenditure, which is determined by three components including physical activity, diet-induced thermogenesis and basal metabolic rate (Levine 2005).

Lower body weight observed in this study in 3xTgAD mice at both 12 and 18 months is not due to decreased food intake, as 3xTgAD mice showed an increase appetite at all ages, which correlates with clinical findings where food intake is adequate or even increased in AD patients (Burns et al 1989; Keene & Hope 1997a; b; Niskanen et al 1993; Spindler et al 1996). In addition to enhanced appetite, 3xTgAD mice exhibited an increased metabolic rate at 12 and 18 months of age. However at this age, 3xTgAD mice were in positive, not negative energy balance, which could be due to the hyperphagia observed at these ages. Consequently, as 3xTgAD mice already weighed less than Non-Tg control mice, a period of negative energy balance could have occurred previously. Evaluation of energy intake and expenditure at more time points throughout the life of 3xTgAD mice would be needed to examine the relationship between energy balance and changes in body weight. It is possible to have weight loss in the presence of positive energy balance as, for example, when there are problems in food absorption in the gut or if there is a reduction in the amount of metabolisable energy that can be obtained from food. The latter can be seen if there is an increase in the energy lost in urine (e.g. altered protein use, impaired kidney function or
diabetes mellitus). As a negative energy balance was not observed in 3xTgAD mice, changes in gut absorption or metabolisable energy could therefore explain the lower body weight seen in these mice after 12 months of age (Knight et al 2010).

3.4.5. Why do aged male 3xTgAD mice have higher metabolic rate?

One reason why aged 3xTgAD mice may have lower body weight than Non-Tg control mice could be a higher metabolic rate, as we see in our mice. An increase in metabolic rate could be caused by factors including raised physical activity, diet-induced thermogenesis or basal metabolic rate.

**Increased physical activity**

At 12 months of age, when 3xTgAD mice were hypermetabolic, they did not show increased general activity within the OF test (see Chapter 4). However, the OF test is not a true test of activity, but rather a test of anxiety, therefore a complete 24 h evaluation of activity remains to be examined in 3xTgAD mice, in order to determine whether raised physical activity plays a role in the hypermetabolic rate seen in 3xTgAD mice at 12 and 18 months of age. Other studies performed in our lab suggest, that at approximately 10 months of age, 3xTgAD mice start to exhibit increased dark phase activity (data unpublished), which could explain the hypermetabolism observed. Similarly Gimenez-Lort and colleagues (2007) found a trend towards increased night/day activity ratio at 6 months of age (Gimenez-Llort et al 2007). In contrast, more recently a study found altered circadian activity in 3xTgAD mice, showing increased locomotor activity during the day and decreased activity at night at both less than 6-months of age and more than 6-months of age (Sterniczuk et al 2010). These studies are interesting, as an increase in physical activity, which is often referred to as “wandering” or “pacing”, is commonly seen in AD patients during the day and at night (Ballard et al 1991; White et al 2004). Raised physical activity has been noted in previous studies in APP and APP/PS-1 mice, which perform a higher total number of moves in the Y-maze SA task from 3 months of age (Holcomb et al 1998; Holcomb et al 1999), show an increase in 24 h home cage activity from 5 months of age (Pugh et al 2007) and move more within an OF from 6 months of age (Dodart et al 1999). Pugh and colleagues also noted that APP/PS-1 mice display no difference in OF behaviour yet mice have decreased body weight despite increased food intake, similar to that seen in the present study (Pugh et al 2007). Increased physical activity may therefore play a role in the increased energy requirement in 3xTgAD mice, however as activity was only examined crudely in these mice in the present study, future work could assess activity in more detail, at different ages and correlate the findings with energy balance.
**Diet–induced thermogenesis**

It is possible that diet-induced thermogenesis could explain the hypermetabolism observed in aged 3xTgAD mice, as these mice consume more food. However, this is unlikely, as food intake is increased in metabolically normal 2-month-old male 3xTgAD mice.

**Increased basal metabolic rate**

The higher metabolic rate observed in male 3xTgAD mice could possibly be due to increased basal metabolic rate, which has been seen in other disorders, including cancer (Bosaeus et al. 2002), burns (Pereira & Herndon 2005), sepsis (Trager et al. 2003) and chronic obstructive pulmonary disease (Schols 2003a; Schols 2003b), where weight loss is a serious complication (Morgan & Gordon 2008; Stephens & Fearon 2008). As with the present study, hypermetabolism in these disorders remains poorly understood. Many factors could increase basal metabolic rate, such as increased whole body protein turnover, and as AD patients show changes in urine protein concentration, this could suggest an increase in protein use (Fonteh et al. 2007). Basal metabolic rate can also be raised by changes in thyroid function (Kapaki et al. 2006) and increases in basal sympathetic nervous system activity (Pascualy et al. 2000), both of which are seen in AD patients. Proton leakage within mitochondrion could also explain increased basal metabolic rate, as mitochondrial damage is seen in AD patients (Baloyannis 2006; Hulbert & Else 2004; Moreira et al. 2006; Morgan & Gordon 2008; Parihar & Brewer 2007). Furthermore, Aβ is seen to make membrane pores (holes) within mitochondrion (Kayed et al. 2004) and has been found localised within mitochondrion (Atamna & Frey 2007; Morgan & Gordon 2008).

**3.4.6 Altered energy balance in AD patients?**

Mounting evidence has demonstrated that AD patients display significant weight loss despite adequate or even increased energy intake (Gillette Guyonnet et al. 2007; Gillette-Guyonnet et al. 2000). It has been hypothesised that higher energy expenditure may explain this weight loss. The existence of a hypermetabolic state has been previously reported in AD patients, who show higher energy requirements than cognitively normal controls, despite lower body weight (Wolf-Klein et al. 1995). However, other studies have shown no change or even a decrease in metabolic rate (Donaldson et al. 1996; Niskanen et al. 1993; Poehlman et al. 1997; Prentice et al. 1989; Wang et al. 1997). The small number of patient studies examining metabolic rate to date have been limited in their sample size, some only describe short-term measurements, whereas others do not have appropriate controls (predicted calculations rather than a control cohort population). It is also possible that many of these patients were not in an active weight loss phase and body weight was stable or between episodes of weight loss. Furthermore, not all AD patients report weight loss (Guerin et al. 2005), therefore energy intake and expenditure would need to be examined from much earlier in the disease in a homogenous population of AD patients who are in a dynamic
phase of weight loss or have previously lost weight. Although a change in metabolism in AD patients is still to be determined, our results in 3xTgAD mice suggest that an increase in metabolic rate may be responsible for the weight loss observed in AD patients.

3.5. Conclusion

AD is a progressive neurodegenerative disorder, characterised by deficits in language, behaviour and memory. Increasing evidence suggests that mid-life obesity is a risk factor for AD, whereas weight loss often occurs before clinical diagnosis and continues during development of the disease. Weight loss may therefore reflect the disease process, and a shift in body weight represents an early indicator of AD, prior to clinical presentation. Mechanisms behind the weight loss are currently unknown, since food intake is usually adequate or even increased in AD patients. Increasing evidence from both preclinical and clinical studies suggest that altered metabolism may contribute to the weight loss observed in AD, however, metabolism has not been previously investigated directly in transgenic mouse models of AD. The data presented in this chapter, therefore, demonstrate for the first time altered body weight, food intake and metabolic rate in the 3xTgAD mouse model of AD, and provide evidence that there is a shift towards a hypermetabolic state from 12 months of age in 3xTgAD mice, which may represent a key stage in advancement of the disease process. However, it remains to be determined when the switch towards a hypermetabolic state occurs. Furthermore, it is unknown if this switch coincides with or is responsible for the lower body weight observed in aged 3xTgAD mice. Future studies could look at more time points to identify when a change in metabolic rate occurs, while also looking at factors which could raise metabolic rate in order to understand the underlying mechanisms.
Chapter 4

Characterisation of the behaviour and memory in 3xTgAD mice
4.1. Introduction and objectives

AD is characterised by progressive deficits in behaviour (Brodaty & Low 2003; Honig & Mayeux 2001; Levy et al 1999; Masterman 2003; Pugh et al 2007; Ritchie & Lovestone 2002) and memory (Artero et al 2003; Carlesimo & Oscar-Berman 1992; Morris 1996; Welsh et al 1992), therefore the second aim of this PhD was to characterise behaviour and memory in cohorts of male Non-Tg control and 3xTgAD mice by using a battery of different behavioural tests, including the open-field (OF), Y-maze spontaneous alternation (SA), odour recognition (OR), novel object recognition (NOR) and Morris water maze (MWM) tests at different ages. This characterisation study was also done to determine baseline behaviour and memory so that future studies could assess the effect of dietary interventions on the onset and progression of cognitive deficits. A separate cohort of C57BL/6 and 129SV mice were also subjected to the OF and SA tasks at different ages to determine any potential strain specific differences in behaviour and memory.

4.2. Materials and methods

4.2.1. Experimental design

4.2.1.1. OF test

To examine anxiety and general activity, separate cohorts of male 3xTgAD and age-matched Non-Tg control mice underwent the OF test (see Method 2.3.1.) at 3 months (Non-Tg, n = 8 and 3xTgAD, n = 7), 5 months (Non-Tg, n = 10 and 3xTgAD, n = 9), and 12 months (Non-Tg, n = 12 and 3xTgAD, n = 11) of age. A cohort of male C57BL/6 (n = 6) and 129SV (n = 6) mice had OF behaviour examined at 3, 5, 12 and 18 months of age.

4.2.1.2. SA test

To examine spatial working memory, separate cohorts of male 3xTgAD and age-matched Non-Tg control mice were submitted to the Y-maze SA test (see Method 2.3.2.) at 3 months (Non-Tg, n = 8 and 3xTgAD, n = 7), 5 months (Non-Tg, n = 10 and 3xTgAD, n = 9), and 12 months (Non-Tg, n = 12 and 3xTgAD, n = 11) of age. A cohort of male C57BL/6 (n = 6) and 129SV (n = 6) mice had SA examined at 3, 5, 12 and 18 months of age.

4.2.1.3. MWM task

To examine spatial memory, separate cohorts of male 3xTgAD and age-matched Non-Tg control mice underwent the MWM task at 3 months (Non-Tg, n = 8 and 3xTgAD, n = 7), 5 months (Non-Tg, n = 8 and 3xTgAD, n = 9), and 12 months (Non-Tg, n = 12 and 3xTgAD, n = 11) of age. The MWM
task consisted of 1 d of flagged platform training, followed by 5 d of submerged platform training and a probe test 24 h after the final day of submerged platform (see Method 2.3.5.)

4.2.1.4. NOR test

In order to examine short-term non-spatial object memory, separate cohorts of male 3xTgAD and age-matched Non-Tg control mice were subjected to the NOR test (see Method 2.3.4.) at 3 months (Non-Tg, n = 12 and 3xTgAD, n = 12) and 8 months (Non-Tg, n = 10 and 3xTgAD, n = 9) of age.

4.2.1.4. OR test

In order to examine short-term smell memory, separate cohorts of male 3xTgAD and age-matched Non-Tg control mice underwent the OR test (see Method 2.3.3.) at 3 months of age (Non-Tg, n = 12 and 3xTgAD, n = 12).

4.2.2. Statistical analysis

Evaluation of OF behaviour, SA, NOR and OR between 3xTgAD and Non-Tg control mice and OF behaviour and SA between C57BL/J6 and 129SV mice were examined using Student's t-tests using Prism (Graphpad, San Diego, CA, USA). MWM training was assessed between cohorts on individual training days and compared within cohorts between the first day of training and successive days of training to assess improvement over time using two-way repeated measures analysis of variance (ANOVA) (SigmaStat 4, Systat Software Inc., IL, USA). Evaluation of the probe test between 3xTgAD and age-matched Non-Tg controls was examined using Student's t-tests using Prism (Graphpad, San Diego, CA, USA).
Chapter 4  Characterisation of behaviour and memory in 3xTgAD mice

4.3. Results

4.3.1. Characterisation of behaviour and memory deficits in 3xTgAD mice

4.3.1.1. 3xTgAD mice displayed age-dependent changes in open-field (OF) behaviour

When placed in an OF arena, 3-month-old male 3xTgAD mice displayed 51% significantly fewer cell entries ($p < 0.01$; Figure 4.1.A), 33% less time moving ($p < 0.001$; Figure 4.1.D) and 66% fewer rearings ($p < 0.001$; Figure 4.1.G) than age-matched Non-Tg control mice. There was no significant difference in the number of defecations between 3xTgAD and age-matched Non-Tg control mice (Figure 4.1.J). When placed in an OF arena, 5-month-old male 3xTgAD mice made 53% significantly fewer cell entries ($p < 0.01$; Figure 4.1.B), spent 37% less time moving ($p < 0.01$, Figure 4.1.E), made 50% fewer rearings ($p < 0.05$; Figure 4.1.H) and had 58% more defecations ($p < 0.05$; Figure 4.1.K) than age-matched Non-Tg control mice. However, at 12 months of age male 3xTgAD mice reared less (38%, $p < 0.01$; Figure 4.1.I) but showed no significant difference in the number of cell entries (Figure 4.1.C), the percentage time moving (Figure 4.1.F) or the number of defecations (Figure 4.1.L) when compared to Non-Tg control mice. Overall, 3xTgAD mice exhibited age-dependent changes in OF behaviour, which included a transient reduction in cell entries and percentage time moving yet rearing exploration was reduced in 3xTgAD mice at all ages tested.

![Figure 4.1. 3xTgAD mice displayed age-dependent changes in open-field (OF) behaviour](image)

Separate cohorts of 3xTgAD ($n = 7-11$) and Non-Tg ($n = 8-12$) control mice were placed in an OF arena and allowed to explore for 5 min at 3, 5 and 12 months of age. The number of cell entries (A-C), percentage time moving (D-F), number of rearings (G-H) and number of defecations (J-L) were recorded. Student’s t-test, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Data are mean ± SEM.
4.3.1.2. 3xTgAD mice showed age-dependent memory deficits in the Y-maze spontaneous alternation (SA) test from 5 months of age

When placed in a Y-maze, 3-month-old male 3xTgAD mice showed no significant difference in the percentage number of alternations (Figure 4.2.A) but performed 34% fewer total number of moves ($p < 0.05$; Figure 4.2.D) when compared to age-matched Non-Tg control mice. By 5 months of age 3xTgAD mice performed a lower (18%) percentage number of alternations ($p < 0.05$; Figure 4.2.B) and also moved less (30%, $p < 0.05$; Figure 4.2.E) when compared to age-matched Non-Tg control mice. When placed in a Y-maze, 12-month-old male 3xTgAD mice also displayed a lower number (23%) of percentage alternations ($p < 0.01$; Figure 4.2.C) but no significant difference in the total number of moves was detected (Figure 4.2.F) when compared to age-matched Non-Tg control mice. Overall, 3xTgAD mice displayed age-dependent memory deficits in the Y-maze SA test from 5 months of age and a transient reduction in activity.

Figure 4.2. 3xTgAD mice showed age-dependent memory deficits in the Y-maze spontaneous alternation (SA) test from 5 months of age

Separate cohorts of 3xTgAD ($n = 7-11$) and Non-Tg ($n = 8-12$) control mice were placed in a Y-maze for 8 min at 3, 5 and 12 months of age. The percentage of spontaneous alternations (A-C) and the total number of moves (D-F) were recorded. Student's t-test, *$p < 0.05$, **$p < 0.01$. Data are mean ± SEM.
4.3.1.3. 3xTgAD mice had age-dependent memory deficits in the Morris water maze (MWM) task probe test from 5 months of age

During flagged platform training, there was no significant difference in escape latency between 3xTgAD and Non-Tg control mice other than at trial two at 3 months, trial four at 5 months and trial one at 12 months of age ($p < 0.05$, Figure 4.3.A-C) where 3xTgAD mice had significantly faster escape latencies. During submerged platform training, Non-Tg control mice showed significant improvement in escape latency over training days at 3 (day 5 versus day 1; $p < 0.05$, Figure 4.3.D), 5 (day 5 versus day 1; $p < 0.05$, Figure 4.3.E) and 12 months of age (day 3-5 versus day 1; $p < 0.05 - p < 0.01$, Figure 4.3.F), whereas 3xTgAD mice showed no evidence of learning at all ages tested. On individual training days there was no significant difference in escape latency between Non-Tg control and 3xTgAD mice at either 3, 5 or 12 months of age. When broken down into a trial-by-trial analysis, no difference in escape latency was seen in 3xTgAD mice at any age between the first trial of a training day and the last trial on the previous day was observed. During the probe test there was no significant difference in the percentage time spent in the target quadrant, where the platform was previously located, between 3xTgAD and Non-Tg control mice at 3 months of age (Figure 4.3.G). However, at both 5 and 12 months of age 3xTgAD showed significant memory deficits during the probe test, indicated by a lower percentage time spent in the target quadrant than Non-Tg control mice (47% and 40% for 5- and 12-month-old mice respectively; $p < 0.001$ and $p < 0.01$, Figure 4.3.H-I). Examination of swim paths revealed most Non-Tg mice at all ages went directly or indirectly to the target quadrant and then spent most of their time in the target quadrant searching for the platform. In contrast most 3xTgAD mice at all ages spent their time equally distributed amongst all the quadrants. At 3 months most 3xTgAD mice appeared to go to the target quadrant then moved away searching elsewhere for the target quadrant. At 5 and 12 months 3xTgAD were more variable with some heading for the target quadrant then swimming elsewhere but most just appeared to swim randomly around the pool. Some appeared to swim in laps around the pool at the same distance from the pool wall to the platform, instead of using the local cues to direct them to the target quadrant. No significant difference in swim speed was noted between Non-Tg control and 3xTgAD mice at all ages tested (data not shown). Overall, 3xTgAD mice displayed normal flagged platform learning yet no improvement in submerged platform training between 3-12 months of age. 3xTgAD mice displayed a significant memory deficit in the probe test from 5 months of age.
Chapter 4  Characterisation of behaviour and memory in 3xTgAD mice

Figure 4.3. 3xTgAD mice had age-dependent memory deficits in the Morris water maze (MWM) probe test from 5 months of age

Separate cohorts of 3xTgAD mice (n = 7-11) and age-matched Non-Tg control mice (n = 8-12) underwent the MWM task at 3, 5 and 12 months of age. Cohorts were given four trials over 1 d of flagged platform training (A-C). Mice were then given four trials a day for 5 d of submerged platform training (D-F). Twenty-four hours after the final trial mice were given a probe test with no platform (G-I). Example swim paths during the probe test are displayed. The green spot indicates the start location and the red dot indicates the end location. The box indicates the location where the platform was located during submerged platform training. For escape latency, two-way repeated measures analysis of variance (ANOVA) with Scheffe post-hoc analysis, *p < 0.05; Non-Tg versus 3xTgAD during flagged platform training, *p < 0.05; Non-Tg versus 3xTgAD during submerged platform training, *p < 0.05; Non-Tg mice day 1 versus days 3-5 of submerged platform training. For percentage time in target quadrant, Student’s t-test; **p < 0.01, ***p < 0.001. Data are mean ± SEM.
4.3.1.4. 3xTgAD mice displayed age-dependent memory deficits in the novel object recognition (NOR) test from 8 months of age

During phase 1, both 3- and 8-month-old male 3xTgAD and age-matched Non-Tg control mice spent a similar percentage of time exploring the identical novel objects 1 and 2 (Figure 4.4.A-B). During phase 2, after an interval of 1 h, both 3-month-old male 3xTgAD and Non-Tg control mice spent a significantly higher percentage of time exploring the novel object than the familiar object, previously presented in phase 1 (45% and 39% respectively; \( p < 0.05 \) Figure 4.4.C). At 8 months of age, during phase 2 of the task, Non-Tg control mice spent a significantly higher percentage of time exploring the novel object than the familiar old object (56%; \( p < 0.01 \), Figure 4.4.D), whereas 3xTgAD mice spent a similar percentage of time exploring both novel and familiar objects, indicating a deficit in memory. Overall, 3xTgAD mice showed age-dependent memory deficits in the NOR test from 8 months of age.

![Figure 4.4](image)

**Figure 4.4.** 3xTgAD mice showed age-dependent memory deficits in the novel object recognition (NOR) test from 8 months of age

Separate cohorts of Non-Tg control and 3xTgAD mice underwent the NOR test at 3 and 8 months of age. During phase 1 (A-B) 3xTgAD (n = 9-12) and Non-Tg (n = 10-12) mice were placed in a NOR arena for 10min with two identical novel objects (novel 1 and novel 2). During phase 2 (C-D), after an interval of 1 h, mice were placed back into the arena with one familiar object (presented in phase 1) and one novel object. Student’s t-test, \( *p < 0.05, **p < 0.01 \). Data are mean ± SEM.
4.3.1.5. 3xTgAD mice showed memory deficits in odour recognition (OR) test at 3 months of age.

During phase 1, both 3-month-old male 3xTgAD and Non-Tg control mice spent a similar percentage of time exploring identically-scented novel balls 1 and 2 (Figure 4.5.A). During phase 2, after an interval of 3 min, 3-month-old male Non-Tg control mice spent a significantly higher percentage of time exploring the novel scented ball than the familiar scented ball previously presented in phase 1 (65%; \( p < 0.001 \) respectively, Figure 4.5.B), whereas 3xTgAD mice spent a similar percentage of time exploring both novel and familiar scented balls. 3xTgAD mice therefore display a memory deficit in the OR test at 3 months of age. It is important to note that male 3xTgAD were able to smell at this age, as they showed an increase in the exploration of female urine over water, streaked on separate pieces of acetate (data not shown).

![Figure 4.5. 3-month-old male 3xTgAD mice displayed memory deficits in the odour recognition (OR) test](image)

*Figure 4.5. 3-month-old male 3xTgAD mice displayed memory deficits in the odour recognition (OR) test*

*During phase 1 (A) 3-month-old 3xTgAD (n = 12) and Non-Tg control mice (n = 12) were placed in OR arena for 10 min with two identically scented novel balls (novel 1 and novel 2). During phase 2 (B), after an interval of 3 min, the mice were placed back into the arena with one familiar scented ball (presented in phase 1) and one novel scented ball. Student’s t-test, **\( p < 0.001 \). Data are mean ± SEM.*
4.3.2. Evaluation of behaviour and memory in 129SV and C57BL/J6 mouse strains.

4.3.2.1. 129SV and C57BL/J6 mice displayed no difference in open field (OF) behaviour at 3, 8, 12 and 18 months of age

When placed in an OF arena, male 129SV and age-matched C57BL/J6 mice displayed no significant difference in the number of cell entries (Figure 4.6. A, E, I & M), percentage time moving (Figure 4.6.B, F, J & N), number of rearings (Figure 4.6.C, G, K & O) and number of defecations (Figure 4.6.D, H, L & P) at 3, 8, 12 and 18 months of age. 129SV and C57BL/J6 mice therefore exhibited similar behaviour in the OF at all ages tested.

129SV (n = 6) and C57BL/J6 (n = 6) mice were placed in an OF arena and allowed to explore for 5 min at 3, 8, 12 and 18 months of age. The number of cell entries (A-D), percentage time moving (E-H), number of rearings (I-L) and the number of defecations (M-P) were recorded. Student’s t-test. Data are mean ± SEM.
4.3.2.2. 129SV and C57BL/J6 mice showed no difference in memory in the Y-maze spontaneous alternation (SA) test at 3, 8, 12 and 18 months of age

When placed in a Y-maze, 129SV and C57BL/J6 mice displayed no significant difference in the percentage number of alternations at 3, 8, 12 or 18 months of age (Figure 4.7.A, C, E & G) No significant difference in the total number of moves was observed between 129SV and C57BL/J6 mice at 3 and 12 months of age, yet a significant difference was observed at 8 and 18 months of age (Figure 4.7.B, D, F & H). Overall, no strain specific differences in memory in Y-maze SA test were observed between 129SV and C57BL/J6 mice at all ages tested.

Figure 4.7. 129SV and C57BL/J6 had no difference in memory in the Y-maze spontaneous alternation (SA) test at 3, 8, 12 and 18 months of age

129SV (n = 6) and C57BL/J6 mice (n = 6) were placed in a Y-maze for 8 min at 3, 8, 12 and 18 months of age. The percentage of spontaneous alternations (A-D) and total number of moves (E-H) were recorded. Student’s t-test, *p < 0.05. Data are mean ± SEM.
4.4. Discussion

Since AD is characterised by progressive deficits in behaviour and memory, the second aim of this PhD was to characterise behaviour and memory deficits in 3xTgAD mice. Behaviour and memory were characterised in male Non-Tg control and 3xTgAD mice in a battery of tests at different ages. 3xTgAD mice displayed altered OF behaviour from 3 months of age, whereas memory was impaired at 3 months of age in the OR test, from 5 months of age in both SA and MWM tests and from 8 months of age in the NOR test. Behaviour and memory were also assessed in 129SV and C57BL/J6 mice (the background strains for the 3xTgAD mice) and no difference was observed between mice at 3, 8, 12 and 18 months of age (apart from a small difference in activity in 8- and 18-month-old mice). Therefore the differences observed here in the 3xTgAD mice are unlikely to be due to a genetic drift towards one of the background strains in these mice.

4.4.1. Open-field (OF) behaviour test

Anxiety, apathy, disinhibition and reduced curiosity all form part of the behavioural and psychological symptoms of dementia (BPSD), which are commonly seen in AD (Daffner et al 1992; Spalletta et al 2004). These symptoms can appear in either the early or late stages of the disease and are known to fluctuate throughout its progression (Cummings 2000; Spalletta et al 2004). Even small stressors, such as a change in routine or environment, are known to play a role in BPSD in AD patients (Smith et al 2004).

In the present study, the OF test was used to evaluate general activity, exploration and anxiety of 3xTgAD mice in response to a novel environment. At 3 and 5 months of age 3xTgAD mice displayed a decrease in activity as the number of cell entries and percentage time moving were reduced when compared to Non-Tg mice, however this difference was no longer seen at 12 months of age. 3xTgAD mice also displayed reduced rearing exploration at 3, 5 and 12 months of age and increased defecation within the OF only at 5 months of age. Thus, overall there was a transient reduction in activity, while rearing was reduced at all ages. Previous studies in 3xTgAD mice have shown normal cell entry activity but less rearing from approximately 2 months of age and cell entry becomes reduced from 6 months of age (Billings et al 2005; Gimenez-Llort et al 2007; Nelson et al 2007). Gimenez-Llort and colleagues also noted increased defecation within the OF at 2.5 and 6 months of age, which is no longer seen at 12 months of age. The results presented in this PhD thesis demonstrate that 3xTgAD mice display changes in OF behaviour in accordance with previous studies. The reason why 3xTgAD mice move around less at 3 and 5 months of age is unknown, but may be due to increased anxiety within the OF. When a mouse is more anxious or unmotivated to explore in a novel environment, it will spend less time moving. While decreased OF activity and rearing exploration are observed at 3 and 5 months of age, defecation within the OF was increased only at 5 months of age. The reason why defecation was not increased at 3 months is unknown. Normal defecation in 3xTgAD mice observed at 12 months is consistent with no
difference in OF activity seen at this age. Increased defecation within an OF has previously been described as anxiety or the inability to cope with mild stressors or novelty (Gimenez-Llort et al 2007). The results from the present study appear to indicate that 3xTgAD mice may be more anxious than Non-Tg control mice, especially at 3 and 5 months of age, and that these differences are less visible in mice by 12 months of age.

Emotional behaviour is dependent on the amygdala, which is known to be affected during early stages of AD by both Aβ and NFTs pathology (Braak & Braak 1991; Espana et al 2010; Hyman et al 1990). Both volumetric and metabolic changes in the amygdala have been associated with BPSD in AD (Bruen et al 2008; Espana et al 2010; Rosen et al 2006). Early changes in OF behaviour and potential increased anxiety are consistent with intraneuronal Aβ pathology observed within the amygdala in the present 3xTgAD colony from 1 month of age (Knight 2007). Other groups have reported intraneuronal Aβ pathology from 2 months of age and NFT pathology from 6 months of age within the amygdala of 3xTgAD mice (Mastrangelo & Bowers 2008). These findings confirm other studies in 3xTgAD mice examining emotional behaviour using other tasks such as the elevated plus maze, the light/dark box or the Boissier 16 hole-board (Gimenez-Llort et al 2007). 3xTgAD mice display innate and conditioned fear at early ages, which the authors correlate with Aβ pathology found specifically within the basolateral amygdala of 3xTgAD (Espana et al 2010).

It has been suggested that stress response and the hypothalamic-pituitary-adrenal axis (HPA) may be altered in 3xTgAD mice (Rothman & Mattson 2009). Endogenous plasma corticosterone (rodent stress hormone) levels are elevated in 3xTgAD mice from 9 months of age (Green et al 2006). However, this is in contrast to the data presented here, where 3xTgAD mice appeared more anxious between 3-5 months of age compared to 12 months of age. Studies have shown that increasing chronic stress by social isolation (Dong et al 2004; Dong et al 2008) or restraint stress (Lee et al 2009) worsen spatial learning and/or increase AD pathology in models of AD. Furthermore, intraperitoneal injection of dexamethasone, a synthetic adrenocortical steroid, increases intraneuronal Aβ and tau in 4-month-old 3xTgAD mice (Green et al 2006). Similarly to AD animal models, dyregulation of the stress response and HPA are observed throughout the progression of AD in humans (Rothman & Mattson 2009). AD patients have higher basal levels of circulating cortisol (human stress hormone) (Davis et al 1986; Masugi et al 1989; Swanwick et al 1998; Umegaki et al 2000) and chronic stress has been shown to worsen cognitive deficits (Rothman & Mattson 2009). However, HPA dysfunction did not worsen as cognition declined further indicating that increased cortisol levels may only be relevant during early disease progression (Swanwick et al 1998).
4.4.1.1. OF behavioural test conclusions and future studies

Results from the OF test indicate that 3xTgAD mice showed age-dependent changes in OF behaviour, correlating with previous studies in 3xTgAD mice and mirroring BPSD observed in AD patients. Future studies would be needed to investigate BPSD-like behaviour in the current colony of Non-Tg control and 3xTgAD mice in more detail. Tests such as the elevated plus maze, the light/dark box, the Boissier 16 hole-board and contextual fear conditioning could be used and correlated with brain pathology and corticosterone levels. It may also be useful to investigate BPSD-like behaviour in 3xTgAD mice over a wider age range, firstly to determine whether 3xTgAD mice are born with altered BPSD-like symptoms and secondly to investigate what happens in mice after 12 months of age when significant plaque pathology is present in the colony.

4.4.2. Y-maze spontaneous alternation (SA) memory test

The predominant feature of AD is memory impairment. During early stages of the disease, short-term or working memory is affected (Huntley & Howard 2010; Ramsden et al 2008). AD patients show deficits in digit and word span tests, which requires patients to remember lists of increasing complexity (Hulme et al 1993). AD patients also show deficits in a number of spatial short-term or working memory tasks, such as the Corsi block-tapping test. In this non-verbal task, cubes are tapped in a novel sequence of increasing difficulty by an examiner, immediately after which participants must reproduce the exact sequence (Fischer 2001; Grossi et al 1993; Guariglia 2007; Hulme et al 1993; Trojano et al 1994). For obvious reasons verbal tests of short-term working memory cannot be used in AD models. The Y-maze SA test, however, is a relevant test of spatial short-term working memory as well as a measure of exploration. SA is dependent on an animal remembering which arms it has previously entered and is thought to be hippocampal-dependent (Douglas 1989; Hughes 2004). The involvement of the hippocampus is supported by studies demonstrating that bilateral or dorsal hippocampal lesions eliminate SA behaviour in rats and mice (Douglas 1989) (Ikonen et al 1999). Furthermore, drugs such as scopolamine, which are known to have amnestic effect on working memory, have been shown to impair SA in rodents (Beninger et al 1986; Sarter et al 1988).

In the present study in male 3xTgAD mice, SA memory was normal at 3 months of age but a memory deficit was observed at 5 and 12 months of age. These results match those observed in other male 3xTgAD mice which display deficits in SA memory at 6 months of age (Zhang et al 2010). Female 3xTgAD mice have normal SA behaviour at 3 and 6 months but they then display deficits at 9-12 months of age (Carroll et al 2007). Several other studies have also reported a reduction in SA in other AD mouse models, with deficits apparent from 3 months of age (Holcomb et al 1998; Hsiao et al 1996; King & Arendash 2002). The SA test is also a measure of exploration; a normal mouse will use the spatial cues around the room to work out an escape route, whereas an
anxious or unmotivated mouse will move around less and not attempt escape. Previous work in 3xTgAD mice has shown that no difference in the total number of moves are observed at 9-12 months of age, when mice showed memory deficits in the Y-maze (Carroll et al 2007). In the present study, while activity was lower at 3 and 5 months of age, there was no significant difference at 12 months of age. These results also support the reduction in activity in the OF, observed in the present and previous studies in 3xTgAD mice (Billings et al 2005; Gimenez-Llort et al 2007).

4.4.2.1. Y-maze SA test conclusions and future studies

The results of the present study demonstrate that 3xTgAD mice show age-dependent deficits in spatial short-term working memory in the Y-maze SA test, confirming previous studies in 3xTgAD mice and in other models of AD, and also mirroring deficits in spatial short-term working memory observed in AD patients. Future studies could investigate what happens in mice after 12 months of age, when significant plaque pathology is present in our colony and examine the onset of SA deficits in female 3xTgAD mice.

4.4.3. Morris water maze (MMW) memory task

AD patients have deficits in spatial memory, including memory for spatial patterns (Sahakian et al 1988), spatial locations (Adelstein et al 1992), spatial paired-associate learning (Swainson et al 2001), object locations within a grid (Bucks & Willison 1997), route learning (Cherrier et al 2001; Kessels et al 2005) and spatial disorientation. Spatial disorientation is defined as a difficulty in orientating and navigating through surroundings and is common in AD (Barrash 1998; Cherrier et al 2001; Henderson et al 1989). During early stages of the disease, AD patients display spatial disorientation in unfamiliar places and, as the disease progresses, disorientation in familiar places often occurs (Cherrier et al 2001).

The MWM task is a relevant spatial memory test, in which mice must learn to use cues to navigate around a pool of cloudy water to find the location of a submerged platform over successive training days (submerged platform training). Twenty-four hours after the final training trial, the platform is removed and the percentage time spent in the target quadrant is assessed (probe trial). This is a test of both short and long-term spatial memory, which is dependent on the hippocampus (Morris et al 1982; Olton & Papas 1979). Studies have shown that lesions to the hippocampus result in a deficit in learning the location of the platform in the MWM task. In AD patients, deficits in spatial memory correlate with changes in hippocampal volume and pathology (Blackwell et al 2004; Kessels et al 2001; Kessels et al 2005; Rodriguez et al 2000).

In the present study, prior to testing memory in the submerged platform trials, mice were subjected to flagged platform training. This is a very important test, as it determines if mice can see properly
and whether mice are able to learn how to use cues and swim towards them to escape onto the platform. It tests whether there are differences in swimming ability and the capability to learn the task between groups of mice. It is also used to minimise stress, as it teaches mice that there is an escape route and hence reduces stress during the subsequent submerged platform training. This part of the test does not involve memory, so no differences between 3xTgAD and control mice should be observed. If 3xTgAD mice displayed difficulty in flagged platform training it would make interpretation of any deficit in submerged platform training difficult. 3xTgAD mice, in this thesis study however, showed no problem in flagged platform learning, consistent with previous studies in 3xTgAD mice (Clinton et al 2007; Zhang et al 2010).

During submerged platform training, Non-Tg control mice showed significant improvement over training days at 3, 5 and 12 months of age, whereas 3xTgAD mice did not show any improvement, suggesting a learning deficit at all ages tested. There was, however, no difference in escape latency between Non-Tg control and 3xTgAD mice on individual training days at all ages. Previous studies have demonstrated that 3xTgAD mice have normal submerged platform training at 2 months of age with memory deficits starting from 4-5 months of age (Billings et al 2005; Clinton et al 2007; Gimenez-Llort et al 2007; Nelson et al 2007; Zhang et al 2010). The reason why 3xTgAD mice, in the present study, did not show memory impairments in training on individual days, when compared to Non-Tg mice, is unknown. In the study by Billings and colleagues, 4-month-old 3xTgAD mice showed no difference in submerged platform training, however when broken down on a trial-by-trial basis, 3xTgAD mice displayed significant deficits in memory retention between the first trial of the day and the last trial of the previous day (Billings et al 2005). When submerged platform training was broken down in the present study, no trial-by-trial differences were observed (data not shown). It was difficult to determine whether cohorts showed learning within a day, as the start position used in the present protocol was sometimes closer to the platform than on other occasions. In order to evaluate learning within submerged training days, future studies could randomise start positions for individual mice, however this is not possible with the current tracking software. During the probe test, 24 h after the final trial of submerged platform training, 3-month-old 3xTgAD mice showed normal memory retention, whereas both 5 and 12-month-old mice displayed significant deficits in memory. These results agree with other studies, in which 3xTgAD mice have normal retention memory in MWM probes at 2 months of age, with onset of deficits occurring from 4-6 months of age (Billings et al 2005; Clinton et al 2007; Zhang et al 2010). Similarly, other models of AD display age-dependent deficits in the MWM from 4 months of age (Chen et al 2000a; Holcomb et al 1999; Hsiao et al 1996; Westerman et al 2002). Swim paths were also assessed in Non-Tg and 3xTgAD mice. There was however variation in paths and platform search strategy between individual animals and it was not possible to break the probe down into time bins, therefore it was difficult to assess differences in detail.
4.4.3.1. MWM task conclusions and future studies

Although there are no directly comparable tests in AD patients to the MWM, it is a relevant test of spatial reference memory. Memory deficits were detected in 3xTgAD mice in the MWM task, matching previous studies in 3xTgAD mice and other models of AD. Future studies could characterise spatial learning in the MWM in more detail, to further understand the deficits observed and to examine the onset of MWM deficits in female 3xTgAD mice. Future studies could also assess swim paths in more detail investigating search strategies of Non-Tg and 3xTgAD mice. The results of individual mice in the MWM could also be correlated with performance in other behavioural tests.

4.4.4. Novel object recognition (NOR) memory test

In screening for AD in humans, there are many tests to evaluate non-spatial visual memory. These tests are meant to screen for deficits in semantic and episodic memory function, thought to be dependent on intact medial temporal lobe function (Egerhazi et al 2007; Lee et al 2003). Both the perirhinal cortex and the hippocampus form part of the medial temporal lobe, which is known to be affected in human AD patients (Squire & Zola-Morgan 1991). In the pattern recognition memory task subjects are presented with a series of 12 visual patterns on a touch sensitive computer screen. Then 20 min after the recognition phase, the subject is required to pick out a pattern previously seen, versus a completely novel one (Egerhazi et al 2007; Owen et al 1995). In the delayed matching to sample task, the subject is shown a pattern for 4.5 s and is instructed to study it, then after a delay of 4 or 12 s the subject must select the previously presented pattern out of a selection of other very similar patterns (Lee et al 2003). The pattern recognition memory and delayed matching to sample tasks are both sensitive to damage in the medial temporal lobe (Egerhazi et al 2007; Lee et al 2003).

The NOR test is a relevant test of non-spatial visual memory in rodents, similar to the pattern recognition and delayed matching to sample tests given to human AD patients. In the NOR test, mice are exposed to two identical objects and, after a set retention time, one of the objects is replaced with a novel one. The NOR test is considered a test of short-term memory that is free from reference memory (need for external cues), making it a non-spatial visual memory test that is based on the natural exploration of novelty in mice (Ennaceur 2010; Ennaceur & Delacour 1988; Ennaceur & Meliani 1992), which can be solved by either recall or familiarity. It is a useful test as it is not dependent on positive or negative reinforcement, such as food or electric shocks. The NOR test appears to be dependent on the perirhinal cortex in rodents (Ennaceur et al 1996), as lesions to the perirhinal cortex causes deficits in object recognition (Ennaceur et al 1996).
In the present study, 3xTgAD mice showed normal memory in the NOR test at 3 months of age but memory was impaired at 8 months of age. In accordance with the present results, previous studies have demonstrated that male 3xTgAD mice display normal memory at 2 and 6 months of age and a deficit at 9 and 15 months of age when the NOR test is performed with a 1.5 h and 24 h delay (Clinton et al 2007). More recently, male 3xTgAD mice exhibit deficits in a 24 h NOR test at 6 months of age (Zhang et al 2010) matching onset of impairments in the NOR test in other models of AD (Dodart et al 1999).

4.4.4.1. NOR test conclusions and future studies

In the present study, 3xTgAD mice showed normal memory in the NOR test at 3 months of age and deficits at 8 months of age. These results are similar to previous work in 3xTgAD and other models of AD; this, together with similarities with some AD screening tests, makes this a useful memory test for 3xTgAD mice. Future studies could look at more inter-trial delays, such as 15 min, 30 min, 4 h, 24 h and 5 d to evaluate short and long term non-spatial visual object memory in more detail. Future studies could also evaluate NOR in female mice, to investigate potential gender differences in non-spatial visual memory.

4.4.5. Odour recognition (OR) task

Impairment in olfactory discrimination is one of the earliest symptoms of AD in humans (Devanand et al 2000; Gilbert et al 2004; Tabert et al 2005). Furthermore, patients with MCI, who present with deficits in working memory together with deficits in olfactory recognition memory, are more likely to progress to AD within 2 years (Devanand et al 2000; Gilbert et al 2004). A commonly used test in AD is the Pennsylvania smell identification test, in which participants who are presented with an odour are then requested to identify the familiar odour amongst other ones not previously presented (Doty et al 1984). AD patients have been shown to have severe deficits in this task early during disease progression (Mesholam et al 1998).

The OR test is a relevant test of short-term smell memory in mice, based on the natural exploration of novelty. At 3 months of age, 3xTgAD mice had a significant deficit in the OR, whereas Non-Tg control mice were able to complete the task. This test demonstrates for the first time that 3xTgAD mice display deficits in a simple smell memory task, at an age when memory impairment is not observed in any other memory task tested here. Thus, the OR test appears to be a sensitive test for detecting early changes in 3xTgAD mice. It is important to note that 3xTgAD mice were able to smell at this age, as they demonstrated an increased exploration of female urine over water (data not shown).

No studies to date have investigated a smell memory in 3xTgAD mice, although limited studies have been performed in other mouse models of AD. APP mice were tested using the novel odour
span task, a non-spatial olfactory working memory task, in which mice are exposed to an increasing number of scents and are required to identify the novel odour (Young et al 2007). When given a 12 scent span test, 3-4-month-old APP mice are unimpaired, whereas when tested with 22 scent span test, they display significant deficits. However, at 8 and 12 months of age, APP mice show significant deficits in both 12 and 22 odour span tests (Young et al 2008). Smell memory deficits have also been reported in other studies using APP mice at 6.5 - 8 months of age (Guerin et al 2009). In this latter study, the authors investigated the exploration time of mice in response to proprinonic acid versus no odour, where mice were food deprived and given food rewards for correct responses (Guerin et al 2009). More recently, APP mice were shown to have normal odour discrimination at 3-4 months of age while deficits were observed after 6 months of age (Wesson et al 2010). Changes in smell memory may be due to pathological changes in the brains of these mice, as degeneration or AD pathology have been observed in areas of the brain involved in smell perception/processing, such as the olfactory bulb, the locus coeruleus, the entorhinal cortex and the piriform cortex (Guerin et al 2009; Jacobsen et al 2006; Kawarabayashi et al 2001; Lehman et al 2003; Wesson et al 2010; Young et al 2008).

Similarly, in AD patients, pathology starts in the perirhinal and entorhinal cortex and spreads out from the medial temporal lobe (Braak & Braak 1995; Slotnick 2001; Young et al 2008). A role for the amygdala in olfactory memory has also been suggested in both rodents and humans (Buchanan et al 2003). Although smell memory has never been assessed in 3xTgAD mice prior to this study, pathology has been noted in relevant brain regions, such as the olfactory bulb, the amygdala and the hippocampus which may explain deficits in OR test observed (Hamilton et al 2010).

4.4.5.1. OR test conclusions and future studies

For the first time, the present study demonstrates that 3xTgAD mice displayed deficits in the OR test at 3 months of age. Interestingly the deficit in the OR test occurs at an age when 3xTgAD mice are unimpaired in the NOR or any other task. This fits in with olfactory discrimination being one of the earliest deficits in AD, making 3xTgAD mice an excellent model to assess smell memory. Deficits in OR, presented here, occur much earlier than reported in other mouse models of AD. Studies in Tg2576, using tasks such as the span task have observed deficits in smell memory from 6 months of age, when deficits start to appear in other tasks, such as the MWM. Tests such as the olfactory span test used in Tg2576 mice have been proven sensitive to investigate smell memory, however these tasks require food restriction or rewards. We have previously demonstrated that 3xTgAD mice are more motivated for food rewards than Non-Tg mice in behavioural tasks (Knight 2007), making odour span tasks not appropriate in this model. Other studies have commented on the potential of confounding factors in traditional odour discrimination tasks (Linster et al 2002; Wesson et al 2008; Wesson et al 2010). Future studies could evaluate OR at more time points in both male and female 3xTgAD mice, examining earlier ages to determine whether 3xTgAD mice
are born with OR deficits and whether deficits are prolonged. Onset of deficits could also be correlated to pathology.

4.4.6. 129SV and C57BL/J6 strains

Behaviour and memory were also assessed in 129SV and C57BL/J6 mice (the background strains for the 3xTgAD mice) and no difference in OF behaviour or SA memory was observed between strains at 3-18 months of age. There was a difference in the total number of moves at 8 and 18 months of age in the Y-maze SA test, however overall it appears that differences in behaviour and memory observed here in 3xTgAD mice were unlikely to be due to a genetic drift towards one of the background strains.

4.5. Conclusion

During the progression of AD, patients display changes in BPSD and memory deficits including those seen in olfactory discrimination, spatial memory and visual memory. The second aim of this PhD study was to characterise behaviour and memory deficits in male Non-Tg control and 3xTgAD mice, in a battery of tests over several ages. The OF, SA, MWM, NOR and OR tests were chosen as they are not reliant on food reinforcers and because they reveal deficits similar to those seen in human AD patients. Using these tests, 3xTgAD mice showed changes in BPSD-like OF behaviour from 3 months of age, earlier than previously reported. Memory impairments are first detected in 3xTgAD mice as deficits in smell memory, which mirror very early deficits in olfactory discrimination seen in AD patients. Deficits in spatial memory were then observed in both the SA and MWM tests from 5 months of age. Finally deficits in non-spatial visual object memory were observed in 3xTgAD mice in the NOR tests at 8 months of age. These results demonstrate that 3xTgAD mice show age-dependent changes in BPSD-like behaviour and memory deficits that are consistent with previous work in 3xTgAD mice, other models of AD and in AD patients. 3xTgAD mice therefore represent a relevant model to evaluate BPSD-like behaviour and memory in response to environmental changes.
Chapter 5

Effect of a high fat diet on 3xTgAD mice
5.1. Introduction and objectives

Emerging evidence suggests that both obesity (Fitzpatrick et al 2009; Rosengren et al 2005; Whitmer et al 2005; Whitmer et al 2008) and the consumption of a high fat (HF) diet are risk factors for AD (Holden 1999; Luchsinger & Mayeux 2004; Luchsinger et al 2002). Furthermore, an HF diet can impair memory or increase pathology in single or double transgenic models of AD (George et al 2004; Ho et al 2004; Kohjima et al 2010; Levin-Allerhand et al 2002; Pedrini et al 2009; Refolo et al 2000; Shie et al 2002). The final aim of this PhD, therefore, was to examine the effect of an HF diet on energy balance, as well as the onset and progression of behaviour and memory deficits in 3xTgAD mice. In order to evaluate whether altering energy balance affects the onset and progression of AD, cohorts of 3xTgAD and age-matched Non-Tg control mice were placed on an HF or standard chow diet. Body weight, food intake, metabolic rate and behaviour were examined in separate cohorts of 3xTgAD and Non-Tg control mice at 3-4, 7-8, 11-12 and 15-16 months of age.

5.2. Materials and methods

5.2.1. Experimental design

5.2.1.1. Energy balance

Cohorts of male 3xTgAD and age-matched Non-Tg mice were fed a chow diet between 4-8 weeks of age. At 8 weeks of age mice were kept on chow or put on an HF diet (see Table 5.1.). Body weight and food intake were measured from weaning until 3-4, 7-8, 11-12 and 15-16 months of age (see Method 2.2.1. - 2.2.2.).

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Monitoring period</th>
<th>Non-Tg/ Chow diet</th>
<th>Non-Tg/ HF diet</th>
<th>3xTgAD/ Chow diet</th>
<th>3xTgAD/ HF diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4 weeks - 3-4 months</td>
<td>n = 11</td>
<td>n = 12</td>
<td>n = 10</td>
<td>n = 10</td>
</tr>
<tr>
<td>2</td>
<td>4 weeks - 7-8 months</td>
<td>n = 12</td>
<td>n = 11</td>
<td>n = 10</td>
<td>n = 10</td>
</tr>
<tr>
<td>3</td>
<td>4 weeks -11-12 months</td>
<td>n = 10</td>
<td>n = 10</td>
<td>n = 10</td>
<td>n = 11</td>
</tr>
<tr>
<td>4</td>
<td>4 weeks -15-16 months</td>
<td>n = 8</td>
<td>n = 9</td>
<td>n =10</td>
<td>n = 11</td>
</tr>
</tbody>
</table>

At the end of each period, the mice (n = 8 for each group) were then housed individually in CLAMS calorimetry cages for 4 days to examine metabolic rate (see Method 2.2.3.). Body weight, caloric intake and water intake were also monitored (see Method 2.2.3.) and 24 h average calculated. All mice were then culled (see Method 2.4.) and epididymal fat pad was weighed.
5.2.1.2. Behaviour and memory

To evaluate the effect of a high fat diet on behaviour and memory in 3xTgAD mice, cohorts (see Table 5.2.) were tested in the Open-field (OF) (see Method 2.3.1.), Y-maze spontaneous alternation (SA) (see Method 2.3.2.), novel object recognition (NOR) (see Method 2.3.4.), odour recognition (OR) (see Method 2.3.3.) and Morris water maze (MWM) (see Method 2.3.5.) tests at 3-4, 7-8, 11-12 and 15-16 months of age. As results from the behaviour and memory characterisation study (see Chapter 4) showed that the percentage of time moving and number of cell entries within the OF were both an indicator of activity and showed the same results, only the number of cell entries made within the OF were assessed in this study. During the MWM task, mice were given 2 d of flagged platform training and 8 d of submerged platform training. As swim paths during the probe test were difficult to interpret with the current software they were not assessed in this study. These tests were performed before measurement of metabolic rate in CLAMS (see 5.2.1.1).

Table 5.2. Experimental cohorts for behavioural evaluation (high fat; HF)

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Age</th>
<th>Non-Tg/ Chow diet</th>
<th>Non-Tg/ HF diet</th>
<th>3xTgAD/ Chow diet</th>
<th>3xTgAD/ HF diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3-4 months</td>
<td><em>n</em> = 11</td>
<td><em>n</em> = 12</td>
<td><em>n</em> = 10</td>
<td><em>n</em> = 10</td>
</tr>
<tr>
<td>2</td>
<td>7-8 months</td>
<td><em>n</em> = 11</td>
<td><em>n</em> = 11</td>
<td><em>n</em> = 10</td>
<td><em>n</em> = 10</td>
</tr>
<tr>
<td>3</td>
<td>11-12 months</td>
<td><em>n</em> = 10</td>
<td><em>n</em> = 10</td>
<td><em>n</em> = 9</td>
<td><em>n</em> = 10</td>
</tr>
<tr>
<td>4</td>
<td>15-16 months</td>
<td><em>n</em> = 8</td>
<td><em>n</em> = 6</td>
<td><em>n</em> = 8</td>
<td><em>n</em> = 10</td>
</tr>
</tbody>
</table>

5.2.2. Statistical analysis

Evaluation of longitudinal body weight between cohorts was examined via three-way repeated measures analysis of variance (ANOVA) with Scheffe post-hoc analysis using Statview 5 (SAS Institute, Cary, NC, USA). Statistical differences between 24 h average body weight, food (caloric) intake, and water intake, and 12 h oxygen consumption (VO₂), carbon dioxide production (VCO₂), respiratory quotient (RQ), energy balance over the 4 d in CLAMS calorimetry cages, OF behaviour and SA between cohorts were examined using two-way ANOVA with Tukey’s post-hoc analysis using JMP (SAS Institute, Cary, NC, USA). Evaluation of NOR and OR was examined using Student’s t-tests (Graphpad, San Diego, CA, USA). MWM training was assessed between cohorts on individual training days and compared within cohorts between the first day of training and successive days of training to assess improvement over time via three-way repeated measures ANOVAs with Scheffe post-hoc analysis using Statview 5 (SAS Institute, Cary, NC, USA). The probe test was analysed using a two-way ANOVA with Tukey’s post-hoc analysis using JMP (SAS Institute, Cary, NC, USA). The epididymal fat pad weight was assessed between cohorts using a two-way ANOVA with Tukey’s post-hoc analysis using JMP (SAS Institute, Cary, NC, USA).
5.3. Results

5.3.1. Effect of an HF diet on energy balance in 3xTgAD mice

5.3.1.1. Effect of an HF diet on body weight in 3xTgAD mice

Cohort 1: weaning to 3-4 months of age

Before changing diets, both groups of 3xTgAD mice weighed more than their respective Non-Tg controls on a chow diet (weeks 6-8, \( p < 0.05 - p < 0.001 \), Figure 5.1.A). The cohort of 3xTgAD mice that were kept on a chow diet continued to weigh more than the Non-Tg chow fed control mice up to 13 weeks of age (\( p < 0.01 - p < 0.001 \)). On an HF diet, the Non-Tg mice weighed more than the Non-Tg mice on a chow diet (weeks 11-13; \( p < 0.01- p < 0.001 \)) reaching up to a similar body weight as 3xTgAD on a chow diet. In contrast, 3xTgAD mice on an HF diet did not weigh more than the 3xTgAD mice on a chow diet for the entire monitoring period. The 3xTgAD mice on an HF diet weighed more than Non-Tg mice on an HF diet only at weeks 10 and 11 (\( p < 0.05 – p < 0.01 \)).

Cohort 2: weaning to 7-8 months of age

Both groups of the 3xTgAD mice weighed more than their respective Non-Tg controls prior to switching diets (weeks 5-8; \( p < 0.05 - p < 0.001 \), Figure 5.1.B). The 3xTgAD mice that were maintained on a chow diet were significantly heavier compared to the Non-Tg controls (on chow) up to 4.5 months of age (\( p < 0.05 - p < 0.001 \)), after which there was no significant difference in body weight between the groups. In contrast, there was no significant difference in body weight between the 3xTgAD and the Non-Tg mice whilst they were maintained on an HF diet. However, 3xTgAD and Non-Tg mice on an HF diet weighed more than their respective chow fed controls (from 3 and 4 months of age for Non-Tg and 3xTgAD mice respectively; \( p < 0.05 – p <0.001 \)).

Cohort 3: weaning to 11-12 months of age

Prior to changing diets, both groups of 3xTgAD mice weighed more than their respective Non-Tg controls on a chow diet (weeks 5-8, \( p < 0.05 - p < 0.01 \), Figure 5.1.C). The cohort of 3xTgAD mice kept on a chow diet weighed more than Non-Tg mice on a chow diet up to 4.5 months of age (\( p < 0.05 - p < 0.001 \)), after which the body weight was not significantly different between the groups. On an HF diet the Non-Tg mice weighed more than the 3xTgAD mice between 4.5 and 8 months of age (\( p < 0.05 - p < 0.001 \)). However, both HF fed 3xTgAD and Non-Tg mice weighed more than their respective controls maintained on a chow diet (from 2.5 and 4.5 months of age for Non-Tg and 3xTgAD mice respectively; \( p < 0.05 – p <0.001 \)).
Chapter 5
Effect of a high fat diet on 3xTgAD mice

Cohort 4: weaning to 15-16 months of age

Both groups of 3xTgAD weighed more than their respective Non-Tg controls on a chow diet prior to changing diets (weeks 5-8, \( p < 0.001 \), Figure 5.1.D). 3xTgAD mice maintained on a chow diet weighed more than Non-Tg mice on a chow diet up to 4.5 months of age (\( p < 0.05 - p < 0.001 \)), after which there was no significant difference in body weight between the groups. In contrast, body weight was not significantly different between the 3xTgAD and Non-Tg mice on an HF diet. However, when maintained on an HF diet, the 3xTgAD and Non-Tg mice weighed more than their respective chow fed controls (from 2 and 4.5 months of age for Non-Tg and 3xTgAD mice respectively; \( p < 0.05 - p < 0.001 \)).

Figure 5.1. Effect of a high fat (HF) diet on body weight in 3xTgAD mice at 3-4, 7-8, 11-12 and 15-16 months of age

Cohorts of male 3xTgAD and age-matched Non-Tg control mice were fed a chow diet between 4-8 weeks of age. At 8 weeks of age cohorts were either kept on chow (Non-Tg; \( n = 8-12 \) and 3xTgAD; \( n = 10 \)) or placed on an HF diet (Non-Tg; \( n = 9-12 \) and 3xTgAD; \( n = 10-11 \)). All cohorts had weekly body weight measured. Repeated measures analysis of variance (ANOVA) with Scheffe post-hoc analysis. *\( p < 0.05 \), **\( p < 0.01 \), ***\( p < 0.001 \) 3xTgAD/chow versus Non-Tg/chow. &\( p < 0.05 \), &&\( p < 0.01 \), &&&\( p < 0.001 \) 3xTgAD/HF versus Non-Tg/HF. #\( p < 0.05 \), ##\( p < 0.01 \), ###\( p < 0.001 \) Non-Tg/HF diet versus Non-Tg/chow. $\ p < 0.05$, $$\ p < 0.01$, $$$\ p < 0.001$ 3xTgAD/HF versus 3xTgAD/chow. Data are mean ± SEM.
5.3.1.2. Effect of an HF diet on energy balance and metabolic rate in 3-4, 7-8, 11-12 and 15-16-month-old 3xTgAD mice in CLAMS metabolic cages

5.3.1.2.1. Effect of an HF diet on body weight, caloric intake and water intake in 3-4-month-old 3xTgAD mice in CLAMS metabolic cages

Two-way ANOVA examining body weight (Figure 5.2.A) revealed that there was an effect of genotype ($F_{3,28} = 23.57, p < 0.001$) and diet ($F_{3,28} = 14.99, p < 0.001$) but no interaction ($F_{3,28} = 2.29, p > 0.05$). Two-way ANOVA examining caloric intake (Figure 5.2.B) revealed that there was an effect of genotype ($F_{3,28} = 4.48, p < 0.05$) and diet ($F_{3,28} = 8.81, p < 0.001$) but no interaction ($F_{3,28} = 1.38, p > 0.05$). Two-way ANOVA examining water intake (Figure 5.2.C) revealed that there was an effect of genotype ($F_{3,28} = 7.9, p < 0.01$) but no effect of diet ($F_{3,28} = 0.95, p > 0.05$) nor any interaction ($F_{3,28} = 1.59, p > 0.05$).

Posthoc analysis revealed that when housed individually in CLAMS calorimetry cages at 3-4 months of age, the 3xTgAD mice on a chow diet showed an increased body weight (14%; $p < 0.001$, Figure 5.2.A), and mean 24h water intake (54%; $p < 0.05$ Figure 5.2.C) but no significant difference in mean 24 h caloric intake (Figure 5.2.B) than the Non-Tg mice on a chow diet. The Non-Tg mice on an HF diet weighed more than the Non-Tg mice on a chow diet (12%; $p < 0.01$) and consumed more calories (36%; $p < 0.05$), whereas an HF diet had no effect on body weight, calorie intake, or water intake in the 3xTgAD when compared to the 3xTgAD mice on a chow diet.
Figure 5.2. Effect of a high fat (HF) diet on body weight, caloric intake and water intake in individually housed 3-4-month-old 3xTgAD mice in Comprehensive Laboratory Animal Monitoring System (CLAMS) cages

Between 4-8 weeks of age cohorts of male triple transgenic 3xTgAD and Non-Tg control mice were fed a chow diet, then at 8 weeks the mice were either kept on chow or placed on an HF diet (n = 8/group). At 3-4 months of age body weight (A), caloric intake (B) and water intake (C) were monitored in individually housed mice over 4 days in CLAMS calorimetry cages. Data are 24h means ± SEM. Two-way analysis of variance (ANOVA) with Tukey’s post-hoc analysis, *p < 0.05, **p < 0.001 3xTgAD/chow versus Non-Tg/chow, #p < 0.05, ##p < 0.01 Non-Tg/HF versus Non-Tg/chow.
5.3.1.2.2. Effect of an HF diet on metabolic rate in 3xTgAD mice at 3-4 months of age

At 3-4 months of age, indirect calorimetry to measure metabolic rate revealed that both the male 3xTgAD and Non-Tg control mice fed a chow diet displayed similar 24 h rhythms in VO₂ consumption (Figure 5.3.A), VCO₂ production (Figure 5.3.B) and RQ (Figure 5.3.C). VO₂, VCO₂ and RQ levels were lowest during the light, largely inactive, phase of the day and highest during the dark, active phase. On an HF diet, both the 3xTgAD and the Non-Tg mice displayed very similar 24 h rhythms in VO₂ consumption (Figure 5.3.A), weak rhythms in VCO₂ production (Figure 5.3.B) and no rhythm in RQ (Figure 5.3.C) when compared to respective chow-fed controls.

Two-way ANOVA examining light phase VO₂ (Figure 5.3.D) revealed that there was an effect of diet (F₃,₂₈ = 4.92, p < 0.05) but no effect of genotype (F₃,₂₈ = 0.26, p > 0.05) nor any interaction (F₃,₂₈ = 3.32, p > 0.05). Two-way ANOVA examining dark phase VO₂ (Figure 5.3.G) revealed that there was an effect of diet (F₃,₂₈ = 38.88, p < 0.001) but no effect of genotype (F₃,₂₈ = 0.02, p > 0.05) nor any interaction (F₃,₂₈ = 2.73, p > 0.05). Two-way ANOVA examining light phase VCO₂ (Figure 5.3.E) revealed that there was an effect of diet (F₃,₂₈ = 48.14, p < 0.001) but no effect of genotype (F₃,₂₈ = 0.45, p > 0.05) nor any interaction (F₃,₂₈ = 3.36, p > 0.05). Two-way ANOVA examining dark phase VCO₂ (Figure 5.3.H) revealed that there was an effect of diet (F₃,₂₈ = 143.4, p < 0.001) but no effect of genotype (F₃,₂₈ = 0.155, p > 0.05) yet there was an interaction between diet and genotype (F₃,₂₈ = 4.55, p < 0.05). Two-way ANOVA examining light phase RQ (Figure 5.3.F) revealed that there was an effect of diet (F₃,₂₈ = 308.5, p < 0.001) but no effect of genotype (F₃,₂₈ = 1.02, p > 0.05) nor any interaction (F₃,₂₈ = 0.18, p > 0.05). Two-way ANOVA examining dark phase RQ (Figure 5.3.I) revealed that there was an effect of diet (F₃,₂₈ = 981.2, p < 0.001) but no effect of genotype (F₃,₂₈ = 1.4, p > 0.05) yet there was an interaction between diet and genotype (F₃,₂₈ = 9.09, p < 0.001).

Posthoc analysis revealed that during the dark phase, the mean 12 h RQ (3%; p < 0.05, Figure 5.3.I) was lower in the 3xTgAD mice than Non-Tg mice fed a chow diet, but there was no difference in any other parameter measured. Compared to a chow diet, the 3xTgAD mice fed an HF diet had lower mean 12 h VCO₂ (18%; p < 0.01, Figure 5.3.E) and lower RQ (14%; p < 0.001, Figure 5.3.F) during the light phase and lower VO₂ (16%; p < 0.05, Figure 5.3.G), CO₂ (32%; p < 0.001, Figure 5.3.H) and RQ (21%; p < 0.001, Figure 5.3.I) during the dark phase. Compared to a chow diet, the Non-Tg mice fed an HF diet displayed lower mean 12 h VO₂ (15% and 26%; p < 0.05 and p < 0.001, Figure 5.3.D-G), VCO₂ (31% and 42%; p < 0.001, Figure 5.3.E-H) and RQ (15% and 24%; p < 0.001, Figure 5.3.F-I) during both light and dark phases. During the metabolic monitoring period, all cohorts of the mice were in positive energy balance. All cohorts exhibited a similar degree of positive energy balance (3xTgAD chow, 4.6 ± 0.7 kcal/day, 3xTgAD HF, 5.7 ± 0.9 kcal/day) except that the Non-Tg mice on an HF diet were in more positive balance than on a chow diet (Non-Tg chow, 2.6 ± 0.7 kcal/day versus Non-Tg HF, 6.2 ± 1.0 kcal/day, p < 0.05).
Figure 5.3. Effect of a high fat (HF) diet on metabolic rate in 3-4-month-old 3xTgAD mice

Between 4-8 weeks of age, cohorts of male triple transgenic 3xTgAD and Non-Tg control mice were fed a chow diet, then at 8 weeks the mice were either kept on chow or placed on an HF diet. At 3-4 months of age cohorts (n = 8 /group) of individually housed mice were placed in Comprehensive Lab Animal Monitoring System (CLAMS) calorimetry cages to examine metabolic rate over four days. Rhythms in VO$_2$ (A) and VCO$_2$ (B) and RQ (C), mean 12 h light phase VO$_2$ (D), VCO$_2$ (E) and RQ (F) and 12 h mean dark phase VO$_2$ (G), VCO$_2$ (H) and RQ (I) were assessed. Data are 12h means ± SEM. Two-way analysis of variance (ANOVA) with Tukey’s post-hoc analysis, *p < 0.05 3xTgAD/chow versus Non-Tg/chow, #p < 0.05, ##p < 0.01 and ###p < 0.001 3xTgAD/HF versus 3xTgAD/chow or Non-Tg/HF versus Non-Tg/chow. (A,B,C), white bars represent the light, inactive phase of the day, and black bars represent the dark active phase.
5.3.1.2.3. Effect of an HF diet on body weight, caloric intake and water intake of 7-8-month-old 3xTgAD mice in CLAMS metabolic cages

Two-way ANOVA examining body weight (Figure 5.4.A) revealed that there was no effect of genotype \( F_{3,28} = 3.36, p > 0.05 \) an effect of diet \( F_{3,28} = 67.27, p < 0.001 \) but no interaction \( F_{3,28} = 0.74, p > 0.05 \). Two-way ANOVA examining caloric intake (Figure 5.4.B) revealed that there was an effect of genotype \( F_{3,28} = 6.89, p < 0.05 \) and diet \( F_{3,28} = 23.7, p < 0.001 \) but no interaction \( F_{3,28} = 0.03, p > 0.05 \). Two-way ANOVA examining water intake (Figure 5.4.C) revealed that there was an effect of genotype \( F_{3,28} = 6.37, p < 0.05 \) and diet \( F_{3,28} = 48.17, p < 0.001 \) but no interaction \( F_{3,28} = 1.15, p > 0.05 \).

Posthoc analysis revealed that when housed individually in CLAMS calorimetry cages at 7-8 months of age, the Non-Tg and the 3xTgAD mice on a HF diet weighed more (55% and 38%; \( p < 0.001 \), Figure 5.4.A), ate more calories (48% and 35%; \( p < 0.01 \) and \( p < 0.05 \), Figure 5.4.B) and drank less water (44% and 47%; \( p < 0.001 \), Figure 5.4.C) than their respective controls on a chow diet. There was no difference in body weight, caloric intake and water intake between the 3xTgAD and the Non-Tg mice on either diet.
Figure 5.4. Effect of a high fat (HF) diet on body weight, caloric intake and water intake in individually housed 7-8-month-old 3xTgAD mice in Comprehensive Laboratory Animal Monitoring System (CLAMS) cages

Between 4-8 weeks of age, cohorts of male triple transgenic 3xTgAD and Non-Tg control mice were fed a chow diet, then at 8 weeks the mice were either kept on chow or placed on an HF diet (n = 8 /group). At 7-8 months of age, body weight (A), caloric intake (B) and water intake (C) were monitored in individually housed mice over four days in CLAMS calorimetry cages. Data are 24 h means ± SEM. Two-way analysis of variance (ANOVA) with Tukey’s post-hoc analysis, #p<0.05, ##p < 0.01, ###p < 0.001 3xTgAD/HF versus 3xTgAD/chow or Non-Tg/HF versus Non-Tg/chow.
5.3.1.2.4. Effect of an HF diet on metabolic rate in 3xTgAD mice at 7-8 months of age

At 7-8 months of age, indirect calorimetry to measure metabolic rate revealed that male 3xTgAD and Non-Tg mice fed a chow diet displayed similar 24 h rhythms in VO₂ consumption (Figure 5.5.A), VCO₂ production (Figure 5.5.B) and RQ (Figure 5.5.C). VO₂, VCO₂ and RQ levels were lowest during the light, largely inactive phase, of the day and highest during the dark, active phase. On an HF diet, 3xTgAD and Non-Tg mice displayed similar 24 h rhythms in VO₂ consumption (Figure 5.5.A), weak rhythms in VCO₂ production (Figure 5.5.B) and no rhythm in RQ (Figure 5.5.C) when compared to their respective controls on a chow diet.

Two-way ANOVA examining light phase VO₂ (Figure 5.5.D) revealed that there was an effect of diet (F₃,₂₈ = 13.05, p < 0.01) but no effect of genotype (F₃,₂₈ = 0.2, p > 0.05) nor any interaction (F₃,₂₈ = 3.95, p > 0.05). Two-way ANOVA examining dark phase VO₂ (Figure 5.5.G) revealed that there was an effect of diet (F₃,₂₈ = 15.01, p < 0.001) but no effect of genotype (F₃,₂₈ = 0.11, p > 0.05) nor any interaction (F₃,₂₈ = 2.27, p > 0.05). Two-way ANOVA examining light phase VCO₂ (Figure 5.5.E) revealed that there was an effect of diet (F₃,₂₈ = 44.84, p < 0.001) but no effect of genotype (F₃,₂₈ = 0.81, p > 0.05) nor any interaction (F₃,₂₈ = 4.83, p > 0.05). Two-way ANOVA examining dark phase VCO₂ (Figure 5.5.H) revealed that there was an effect of diet (F₃,₂₈ = 70.35, p < 0.001) but no effect of genotype (F₃,₂₈ = 0.71, p > 0.05) yet there was an interaction between diet and genotype (F₃,₂₈ = 4.69, p < 0.05). Two-way ANOVA examining light phase RQ (Figure 5.5.F) revealed that there was an effect of diet (F₃,₂₈ = 128.3, p < 0.001) but no effect of genotype (F₃,₂₈ = 0.24, p > 0.05) nor any interaction (F₃,₂₈ = 0.69, p > 0.05). Two-way ANOVA examining dark phase RQ (Figure 5.5.I) revealed that there was an effect of diet (F₃,₂₈ = 253.8, p < 0.001) but no effect of genotype (F₃,₂₈ = 1.18, p > 0.05) nor any interaction (F₃,₂₈ = 0.63, p > 0.05).

Posthoc analysis revealed that there was no difference in mean 12 h light or dark VO₂, VCO₂ and RQ between the Non-Tg and the 3xTgAD mice on a chow diet (Figure 5.5.D-I). Compared to a chow diet, 3xTgAD mice fed an HF diet displayed lower mean 12 h VCO₂ (18% and 23%; p < 0.01 and p < 0.001, Figure 5.5.E & H) and RQ (16% and 19%; p < 0.001, Figure 5.5.F & I) during the light and dark phases. However, in addition to a reduction in 12 h VCO₂ (32% and 36%; p < 0.001, Figure 5.3.E-H) and RQ (14% and 21%; p < 0.001, Figure 5.3.F-I), the Non-Tg mice fed an HF diet also had lower VO₂ (19% and 7%; p < 0.01, Figure 5.5.D & G) compared to chow-fed Non-Tg mice. During the metabolic monitoring period, all cohorts of mice exhibited a similar degree of positive energy balance (Non-Tg chow, 6.5 ± 1.3 kcal/day, Non-Tg HF, 12.4 ± 2.3 kcal/day, 3xTgAD chow, 10.0 ± 1.6 kcal/day and 3xTgAD HF, 15.9 ± 1.5 kcal/day, p > 0.05).
Figure 5.5. Effect of a high fat (HF) diet on metabolic rate in 7-8-month-old 3xTgAD mice

Between 4-8 weeks of age cohorts of male triple transgenic 3xTgAD and Non-Tg control mice were fed a chow diet, then at 8 weeks the mice were either kept on chow or placed on an HF diet. At 7-8 months of age cohorts (n = 8/group) were placed in Comprehensive Lab Animal Monitoring System (CLAMS) calorimetry cages to examine metabolic rate. Rhythms in VO_{2} (A) and VCO_{2} (B) and RQ (C), mean 12 h light phase VO_{2} (D), VCO_{2} (E) and RQ (F) and 12 h mean dark phase VO_{2} (G), VCO_{2} (H) and RQ (I) were assessed. Data are 12 h means ± SEM. Two-way analysis of variance (ANOVA) with Tukey’s post-hoc analysis, ##p < 0.01 and ###p < 0.001 3xTgAD/HF versus 3xTgAD/chow or Non-Tg/HF versus Non-Tg/chow. (A, B, C), white bars represent the light, inactive phase of the day, and black bars represent the dark active phase.
5.3.1.2.5. Effect of an HF diet on body weight, caloric intake and water intake of 11-12-month-old 3xTgAD mice individually housed in CLAMS metabolic cages

Two-way ANOVA examining body weight (Figure 5.6.A) revealed that there was no effect of genotype ($F_{3,28} = 1.68, p > 0.05$), an effect of diet ($F_{3,28} = 123.7, p < 0.001$) but no interaction ($F_{3,28} = 0.04, p > 0.05$). Two-way ANOVA examining caloric intake (Figure 5.6.B) revealed that there was no effect of genotype ($F_{3,28} = 0.42, p > 0.05$), an effect of diet ($F_{3,28} = 10.26, p < 0.001$) but no interaction ($F_{3,28} = 0.15, p > 0.05$). Two-way ANOVA examining water intake (Figure 5.6.C) revealed that there was an effect of genotype ($F_{3,28} = 9.02, p < 0.01$) and diet ($F_{3,28} = 16.31, p < 0.001$) but no interaction ($F_{3,28} = 1.39, p > 0.05$).

Posthoc analysis revealed when housed individually in CLAMS calorimetry cages at 11-12 months the 3xTgAD mice on a chow diet drank more (31%; $p < 0.05$ Figure 5.6.C) than the Non-Tg on a chow diet, but no difference in body weight and caloric intake were observed. On an HF diet, both the Non-Tg and 3xTgAD weighed more (74% and 72%; $p < 0.001$, Figure 5.6.A) yet displayed no difference in caloric intake (Figure 5.6.B) than their respective controls on a chow diet. The 3xTgAD mice on an HF drank less than the 3xTgAD mice on a chow diet (40%; $p < 0.01$ Figure 5.6.C).
Figure 5.6. Effect of a high fat (HF) diet on body weight, caloric intake and water intake in 11-12-month-old 3xTgAD mice individually housed in CLAMS metabolic cages

Between 4-8 weeks of age, cohorts of male triple transgenic 3xTgAD and Non-Tg mice were fed a chow diet, then at 8 weeks the mice were either kept on chow or placed on an HF diet (n = 8/group). At 11-12 months of age, body weight (A), caloric intake (B) and water intake (C) were monitored in individually housed mice over four days in Comprehensive Laboratory Animal Monitoring System (CLAMS) calorimetry cages. Data are 24 h means ± SEM. Two-way analysis of variance (ANOVA) with Tukey’s post-hoc analysis, *p < 0.05 3xTgAD/chow versus Non-Tg/chow, ##p < 0.01, ###p < 0.001 3xTgAD/HF versus 3xTgAD/chow or Non-Tg/HF versus Non-Tg/chow.
5.3.1.2.6. Effect of an HF diet on metabolic rate in 3xTgAD mice at 11-12 months of age

At 11-12 months of age, indirect calorimetry to measure metabolic rate revealed that both the male 3xTgAD and Non-Tg mice fed a chow diet displayed similar 24 h rhythms in VO$_2$ consumption (Figure 5.7.A), VCO$_2$ production (Figure 5.7.B) and RQ (Figure 5.7.C). VO$_2$, VCO$_2$ and RQ levels were lowest during the light, largely inactive phase of the day and highest during the dark, active phase. On an HF diet, both the 3xTgAD and Non-Tg mice displayed very similar 24 h rhythms in VO$_2$ consumption (Figure 5.7.A), weak rhythms in VCO$_2$ production (Figure 5.7.B) and no rhythm in RQ (Figure 5.7.C) when compared to their respective chow-fed controls.

Two-way ANOVA examining light phase VO$_2$ (Figure 5.7.D) revealed that there was an effect of diet ($F_{3,28} = 89.69, p < 0.001$) but no effect of genotype ($F_{3,28} = 0.05, p > 0.05$) nor any interaction ($F_{3,28} = 0.89, p > 0.05$). Two-way ANOVA examining dark phase VO$_2$ (Figure 5.7.G) revealed that there was an effect of diet ($F_{3,28} = 94.79, p < 0.001$) but no effect of genotype ($F_{3,28} = 0.5, p > 0.05$) nor any interaction ($F_{3,28} = 0.37, p > 0.05$). Two-way ANOVA examining light phase VCO$_2$ (Figure 5.7.E) revealed that there was an effect of diet ($F_{3,28} = 125.05, p < 0.001$) but no effect of genotype ($F_{3,28} = 2.15, p > 0.05$) nor any interaction ($F_{3,28} = 3.77, p > 0.05$). Two-way ANOVA examining dark phase VCO$_2$ (Figure 5.7.H) revealed that there was an effect of diet ($F_{3,28} = 151.5, p < 0.001$) but no effect of genotype ($F_{3,28} = 0.003, p > 0.05$) nor any interaction ($F_{3,28} = 0.76, p > 0.05$). Two-way ANOVA examining light phase RQ (Figure 5.7.F) revealed that there was an effect of diet ($F_{3,28} = 84.79, p < 0.001$) and genotype ($F_{3,28} = 8.73, p < 0.01$) and a significant interaction ($F_{3,28} = 7.34, p < 0.05$). Two-way ANOVA examining dark phase RQ (Figure 5.7.I) revealed that there was an effect of diet ($F_{3,28} = 250.85, p < 0.001$) but no effect of genotype ($F_{3,28} = 2.06, p > 0.05$) nor any interaction ($F_{3,28} = 1.65, p > 0.05$).

Posthoc analysis revealed that there was no difference in mean 12 h light or dark VO$_2$, VCO$_2$ and RQ between the Non-Tg and 3xTgAD mice on a chow diet (Figure 5.7.D-I). Mean 12 h VO$_2$ ($p < 0.001$, Figure 5.7.D & G), VCO$_2$ ($p < 0.001$ Figure 5.7.E & H) and RQ ($p < 0.001$ Figure 5.7.F & I) were lower during the light and dark phases in both HF fed 3xTgAD and Non-Tg mice, when compared to respective controls on a chow diet. During the metabolic monitoring period, all cohorts of mice exhibited a similar degree of positive energy balance (Non-Tg chow, 6.4 ± 1.0 kcal/day, Non-Tg HF, 12.2 ± 2.8 kcal/day, 3xTgAD chow, 7.9 ± 0.8 kcal/day and 3xTgAD HF, 11.5 ± 2.8 kcal/day, $p > 0.05$).
**Figure 5.7. Effect of a high fat (HF) diet on metabolic rate in 11-12-month-old 3xTgAD mice**

Between 4-8 weeks of age cohorts of male triple transgenic 3xTgAD and Non-Tg control mice were fed a chow diet, then at 8 weeks the mice were either kept on chow or placed on an HF diet. At 11-12 months of age cohorts (Non-Tg/chow; n = 8, Non-Tg/HF; n = 4, 3xTgAD/chow; n = 7 and 3xTgAD/HF; n = 8) were placed in Comprehensive Lab Animal Monitoring System (CLAMS) calorimetry cages for four days to examine metabolic rate. Rhythms in VO$_2$ (A) and VCO$_2$ (B) and RQ (C), mean 12 h light phase VO$_2$ (D), VCO$_2$ (E) and RQ (F) and 12 h mean dark phase VO$_2$ (G), VCO$_2$ (H) and RQ (I) were assessed. Data are 12 h means ± SEM. Two-way analysis of variance (ANOVA) with Tukey’s post-hoc analysis, ###p < 0.001 3xTgAD/HF versus 3xTgAD/chow or Non-Tg/HF versus Non-Tg/chow. (A, B, C), white bars represent the light, inactive phase of the day, and black bars represent the dark active phase.
5.3.1.2.7. Effect of an HF diet on body weight, caloric intake and water intake in 15-16-months-old 3xTgAD mice individually housed in CLAMS metabolic cages

Two-way ANOVA examining body weight (Figure 5.8.A) revealed that there was no effect of genotype ($F_{3,23} = 0.24, p > 0.05$), an effect of diet ($F_{3,23} = 72.87, p < 0.001$) but no interaction ($F_{3,23} = 0.43, p > 0.05$). Two-way ANOVA examining caloric intake (Figure 5.8.B) revealed that there was no effect of genotype ($F_{3,23} = 1.61, p < 0.05$), an effect on diet ($F_{3,23} = 22.93, p < 0.001$) but no interaction ($F_{3,23} = 1.16, p > 0.05$). Two-way ANOVA examining water intake was not significant (Figure 5.8.C).

Posthoc analysis revealed that when housed individually in CLAMS calorimetry cages at 15-16 months of age, Non-Tg control and 3xTgAD mice on an HF diet weighed more (53% and 63%; $p < 0.001$, Figure 5.8.A), consumed more calories (70% and 34%; $p < 0.001$ and $p > 0.05$, Figure 5.8.B) but showed no difference in water intake when compared to their respective controls on a chow diet (Figure 5.8.C). There was no difference in body weight, caloric intake or water intake between the 3xTgAD and Non-Tg mice on either diet.
Figure 5.8. Effect of a high fat (HF) diet on body weight, caloric intake and water intake in 15-16-month-old 3xTgAD mice individually housed in CLAMS metabolic cages.

Between 4-8 weeks of age, cohorts of male triple transgenic 3xTgAD and Non-Tg mice were fed a chow diet, then at 8 weeks the mice were either kept on chow or placed on an HF diet. At 15-16 months of age cohorts (Non-Tg/chow diet; n = 8, Non-Tg/HF; n = 4, 3xTgAD/chow; n = 7 and 3xTgAD/HF; n = 8) had body weight (A), caloric intake (B) and water intake (C) monitored when individually housed over four days in Comprehensive Laboratory Animal Monitoring System (CLAMS) calorimetry cages. Two-way analysis of variance (ANOVA) with Tukey’s post-hoc analysis, #p < 0.05, ###p < 0.001 3xTgAD/HF versus 3xTgAD/chow or Non-Tg/HF versus Non-Tg/chow.
5.3.1.2.8. Effect of an HF diet on metabolic rate in 15-16-month-old 3xTgAD mice

At 15-16 months of age, indirect calorimetry to measure metabolic rate revealed that both the male 3xTgAD and Non-Tg mice on a chow diet displayed similar 24 h rhythms in VO$_2$ consumption (Figure 5.9.A), VCO$_2$ production (Figure 5.9.B) and RQ (Figure 5.9.C). VO$_2$, VCO$_2$ and RQ levels were lowest during the light, largely inactive phase of the day and highest during the dark, active phase. On an HF diet, both the 3xTgAD and Non-Tg mice had similar 24 h rhythms in VO$_2$ consumption (Figure 5.9.A), weak rhythms in VCO$_2$ production (Figure 5.9.B) and no rhythm in RQ (Figure 5.9.C) compared to their respective controls on a chow diet.

Two-way ANOVA examining light phase VO$_2$ (Figure 5.9.D) revealed that there was an effect of diet ($F_{3,23} = 13.69, p < 0.01$) but no effect of genotype ($F_{3,23} = 0.26, p > 0.05$) nor any interaction ($F_{3,23} = 0.05, p > 0.05$). Two-way ANOVA examining dark phase VO$_2$ (Figure 5.9.G) revealed that there was an effect of diet ($F_{3,23} = 1.23, p < 0.001$) but no effect of genotype ($F_{3,23} = 21.31, p > 0.05$) nor any interaction ($F_{3,23} = 0.5, p > 0.05$). Two-way ANOVA examining light phase VCO$_2$ (Figure 5.9.E) revealed that there was an effect of diet ($F_{3,23} = 104.3, p < 0.001$), but no effect of genotype ($F_{3,23} = 23.77, p > 0.05$) nor any interaction ($F_{3,23} = 2.53, p > 0.05$). Two-way ANOVA examining dark phase VCO$_2$ (Figure 5.9.H) revealed that there was an effect of diet ($F_{3,23} = 186.88, p < 0.001$), genotype ($F_{3,23} = 9.74, p < 0.01$) and a significant interaction ($F_{3,23} = 5.04, p < 0.05$). Two-way ANOVA examining light phase RQ (Figure 5.9.F) revealed that there was an effect of diet ($F_{3,23} = 108.86, p < 0.001$), genotype ($F_{3,23} = 34.42, p < 0.001$) and a significant interaction ($F_{3,23} = 18.44, p < 0.001$). Two-way ANOVA examining dark phase RQ (Figure 5.9.I) revealed that there was an effect of diet ($F_{3,23} = 460.2, p < 0.001$) but no effect of genotype ($F_{3,23} = 0.28, p > 0.05$) nor any interaction ($F_{3,23} = 1.26, p > 0.05$).

Posthoc analysis revealed that during the light phase, the mean 12 h RQ was reduced (13%; $p < 0.001$, Figure 5.9.F) and during the dark phase VCO$_2$ was significantly increased (14%; $p < 0.01$, Figure 5.9.H) in the 3xTgAD, compared to Non-Tg mice on a chow diet. The increase in VCO$_2$ in chow fed 3xTgAD mice is suggestive of an increase in metabolic rate. No difference in light phase VO$_2$ was observed (Figure 5.9.D), whereas dark phase VO$_2$ was significantly lower in both the HF fed 3xTgAD and Non-Tg mice, compared to their respective chow fed controls ($p < 0.05 - p < 0.01$, Figure 5.3.G). In contrast, both mean 12 h VCO$_2$ ($p < 0.01 - p < 0.001$, Figure 5.9.E & H) and RQ ($p < 0.001$, Figure 5.9.F & I) were lower during the light and dark phases in HF fed 3xTgAD and Non-Tg mice when compared to respective controls on a chow diet. During the metabolic monitoring period, all cohorts of mice were in positive energy balance. All cohorts exhibited a similar degree of positive energy balance (3xTgAD chow, 6.7 ± 0.8 kcal/day, 3xTgAD HF, 10.4 ± 1.9 kcal/day) except that the Non-Tg mice on an HF diet were in more positive balance than on a chow diet (Non-Tg chow, 3.8 ± 0.7 kcal/day versus Non-Tg HF, 11.0 ± 2.8 kcal/day, $p < 0.05$).
Figure 5.9. Effect of a high fat (HF) diet on metabolic rate in 15-16-month-old 3xTgAD mice

Between 4-8 weeks of age cohorts of male triple transgenic 3xTgAD and Non-Tg control mice were fed a chow diet, then at 8 weeks the mice were either kept on chow or placed on an HF diet. At 15-16 months of age cohorts (Non-Tg/ chow; n = 8, Non-Tg/HF; n = 4, 3xTgAD/chow; n = 7 and 3xTgAD/HF; n = 8) were placed in Comprehensive Lab Animal Monitoring System (CLAMS) calorimetry cages to examine metabolic rate. Rhythms in VO$_2$ (A) and VCO$_2$ (B) and RQ (C), mean 12 h light phase VO$_2$ (D), VCO$_2$ (E) and RQ (F) and 12 h mean dark phase VO$_2$ (G), VCO$_2$ (H) and RQ (I) were assessed. Data are 12 h means ± SEM. Two-way analysis of variance (ANOVA) with Tukey’s post-hoc analysis, **p < 0.01, ***p < 0.001 3xTgAD/chow mice versus Non-Tg /chow, #p < 0.05, ##p < 0.01 and ###p < 0.001 3xTgAD/HF versus 3xTgAD/chow or Non-Tg/HF versus Non-Tg/chow. (A, B, C), white bars represent the light, inactive phase of the day, and black bars represent the dark active phase.
5.3.1.3. Effect of an HF diet on epididymal fat pad weight in 3xTgAD mice at 3-4, 7-8, 11-12 and 15-16 months of age

Two-way ANOVA examining epididymal fat pad weight at 3-4 months of age (Figure 5.10.A), revealed that there was an effect of diet ($F_{3,39} = 91.37, p < 0.001$) and genotype ($F_{3,39} = 12.22, p < 0.01$) but no interaction ($F_{3,39} = 4.06, p > 0.05$). Two-way ANOVA examining epididymal fat pad weight at 7-8 months of age (Figure 5.10.B), revealed that there was an effect of diet ($F_{3,38} = 72.09, p < 0.001$), genotype ($F_{3,38} = 5.33, p < 0.05$) and a significant interaction ($F_{3,38} = 6.85, p < 0.05$). Two-way ANOVA examining epididymal fat pad weight at 11-12 months of age (Figure 5.10.C), revealed that there was an effect of diet ($F_{3,34} = 73.31, p < 0.001$), genotype ($F_{3,34} = 27.61, p < 0.001$) and a significant interaction ($F_{3,34} = 34.98, p < 0.001$). Two-way ANOVA examining epididymal fat pad weight at 11-12 months of age (Figure 5.10.D), revealed that there was an effect of diet ($F_{3,24} = 23.3, p < 0.001$), genotype ($F_{3,24} = 12.47, p < 0.01$) and a significant interaction ($F_{3,24} = 20.87, p < 0.001$).

Posthoc analysis revealed that at 3-4, 7-8, 11-12 and 15-16 months of age the male 3xTgAD mice on a chow diet displayed no difference in epididymal fat pad weight compared to the chow fed Non-Tg mice (Figure 5.10. A-D). However, HF feeding lead to an increase (168% - 407%; $p < 0.001$, Figure 5.10.A-B) in epididymal fat pad weight in both the 3xTgAD and Non-Tg mice at 3-4 and 7-8 months of age compared to their respective controls on a chow diet. Epididymal fat pad weight was lower in the 3xTgAD than the Non-Tg mice on an HF diet at 3-4 months of age (31%; $p < 0.01$) and higher in 3xTgAD mice at 7-8 months of age (55%; $p < 0.01$). At 11-12 and 15-16 months of age, epididymal fat pad weight was not different in Non-Tg mice on an HF diet, whereas it was higher in the 3xTgAD mice on an HF diet (725%-778%; $p < 0.001$, Figure 5.10.C-D) compared to their respective controls on a chow diet.
Chapter 5  Effect of a high fat diet on 3xTgAD mice

Figure 5.10. Effect of high fat (HF) diet on epididymal fat pad weight in 3xTgAD mice at 3-4, 7-8, 11-12 and 15-16 months of age

Between 4-8 weeks of age, cohorts of male 3xTgAD and age-matched Non-Tg mice were fed a chow diet, then at 8 weeks the mice were either kept on chow (Non-Tg; n = 8-12 and 3xTgAD; n = 10) or placed on an HF diet (Non-Tg; n =9-12 and 3xTgAD; n = 10-11). At 3-4 (A), 7-8 (B), 11-12 (C) and 15-16 (D) months of age epididymal fat pad weight was measured. Data are 24 h means ± SEM. Two-way analysis of variance (ANOVA) with Tukey's post-hoc analysis, **p < 0.01, ***p < 0.001 3xTgAD/HF versus Non-Tg/HF, ###p < 0.001 3xTgAD/HF versus 3xTgAD/chow or Non-Tg/HF versus Non-Tg/chow.
5.3.2. Effect of an HF diet on behaviour and memory in 3xTgAD mice at 3-4, 7-8, 11-12 and 15-16 months of age

5.3.2.1. Effect of an HF diet on open-field (OF) behaviour in 3xTgAD mice at 3-4, 7-8, 11-12 and 15-16 months of age

Two-way ANOVA examining the number of cell entries at 3-4 months of age (Figure 5.11.A), revealed an effect of genotype ($F_{3,39} = 0.04, p < 0.001$) but no effect of diet ($F_{3,39} = 28.82, p > 0.05$) nor any interaction ($F_{3,39} = 0.08, p > 0.05$). Two-way ANOVA examining the number of cell entries at 7-8 months of age (Figure 5.11.B), revealed an effect of genotype ($F_{3,38} = 27.97, p < 0.001$) but no effect of diet ($F_{3,38} = 0.02, p > 0.05$) nor any interaction ($F_{3,38} = 0.05, p > 0.05$). Two-way ANOVA examining the number of cell entries at 11-12 months of age (Figure 5.11.C) and 15-15 months of age (Figure 5.11.D) was not significant. Two-way ANOVA examining the number of rearings at 3-4 months of age (Figure 5.11.E), revealed an effect of genotype ($F_{3,39} = 93.67, p < 0.001$), no effect of diet ($F_{3,39} = 2.48, p > 0.05$) yet there was a significant interaction ($F_{3,39} = 4.21, p < 0.05$). Two-way ANOVA examining the number of rearings at 7-8 months of age (Figure 5.11.F), revealed an effect of genotype ($F_{3,38} = 17.92, p < 0.001$) but no effect of diet ($F_{3,38} = 0.009, p > 0.05$) nor any interaction ($F_{3,38} = 0.61, p > 0.05$). Two-way ANOVA examining the number of rearings at 11-12 months and 15-15 months of age was not significant (Figure 5.11.G-H). Two-way ANOVA examining the number of defecations at all ages was not significant (Figure 5.11.I-L).

Posthoc analysis revealed that when placed in an OF arena, 3-4-month-old 3xTgAD mice on a chow or HF diet performed fewer cell entries (38% and 44%; $p < 0.01$, Figure 5.11.A), and rearings (66% and 54%; $p < 0.001$, Figure 5.11.E) but no difference in the number of defecations (Figure 5.11.I) was observed when compared to their respective Non-Tg controls on the chow or HF diet. However, HF feeding had no effect on the behaviour of the Non-Tg or 3xTgAD mice at 3-4 months of age. At 7-8 months of age, 3xTgAD mice on a chow diet performed less cell entries (66%; $p < 0.001$, Figure 5.11.B) and number of rearings (61%; $p < 0.01$, Figure 5.11.F), yet no difference in the number of defecations (Figure 5.11.J) was detected when compared to chow-fed Non-Tg mice. Diet had no effect on behaviour in 7-8-month-old Non-Tg mice, whereas HF feeding reversed the reduction in cell entries observed in chow fed 3xTgAD mice (122%; $p < 0.05$). At 11-12 and 15-16 months of age, there was no effect of genotype or diet on the number of cell entries (Figure 5.11.C-D), rearings (Figure 5.11.G-H) or defecations (Figure 5.11.K-L).
Chapter 5  
Effect of a high fat diet on 3xTgAD mice

Figure 5.11. Effect of high fat (HF) diet on open-field (OF) behaviour in 3xTgAD mice at 3-4, 7-8, 11-12 and 15-16 months of age

Between 4-8 weeks of age cohorts of male triple transgenic 3xTgAD and age-matched Non-Tg mice were fed a chow diet, then at 8 weeks the mice were either kept on chow or placed on an HF diet. At 3-4, 7-8, 11-12 and 15-16 months of age cohorts (Non-Tg/chow, n= 8-11, Non-Tg/HF; n =6-12, 3xTgAD/chow; n = 8-10 and 3xTgAD/HF; n = 10) were placed in an OF arena and allowed to explore for 5 min. Cell entries (A-D), rearings (E-H) and number of defecations (I-L) were measured. Data are 24 h means ± SEM. Two-way analysis of variance (ANOVA) with Tukey’s post-hoc analysis, **p < 0.01, ***p < 0.001 3xTgAD/chow versus Non-Tg/chow, and 3xTgAD/HF versus Non-Tg/HF #p<0.05, 3xTgAD/chow versus 3xTgAD/HF
5.3.2.2. Effect of an HF diet on Y-maze spontaneous alternation (SA) in 3xTgAD mice at 3-4, 7-8, 11-12 and 15-16 months of age

Two-way ANOVA examining percentage alternation at 3-4 months of age was not significant (Figure 5.12.A). Two-way ANOVA examining percentage alternation at 7-8 months of age (Figure 5.12.B), revealed an effect of diet \( F(3,38) = 0.03, p < 0.01 \) but no effect of genotype \( F(3,38) = 12.46, p > 0.05 \) nor any interaction \( F(3,38) = 42.76, p > 0.05 \). Two-way ANOVA percentage alternation at 11-12 months of age (Figure 5.12.C), revealed an effect of diet \( F(3,35) = 13.82, p < 0.001 \), genotype \( F(3,35) = 4.34, p < 0.05 \) and a significant interaction \( F(3,35) = 4.14, p < 0.05 \). Two-way ANOVA examining percentage alternation at 15-16 months of age (Figure 5.12.D), revealed an effect of diet \( F(3,27) = 32.71, p < 0.001 \), genotype \( F(3,27) = 14.59, p < 0.001 \) but no interaction \( F(3,27) = 1.91, p > 0.05 \).

Posthoc analysis revealed that when placed in a Y-maze at 3-4 months of age, there was no effect of diet or genotype on percentage alternations (Figure 5.12.A), but the 3xTgAD mice fed either a chow or HF diet showed a reduction in the total number of moves compared to their respective controls (31% and 35%; \( p < 0.05 \) and \( p < 0.01 \), Figure 5.12.E). At 7-8 months of age, the 3xTgAD mice on a chow diet displayed no difference in percentage alternation (Figure 5.12.B) yet they performed fewer moves than Non-Tg mice on a chow diet (32%; \( p < 0.05 \), Figure 5.12.F). However, there was no significant difference in percentage alternation or number of moves in 3xTgAD mice fed an HF diet compared to Non-Tg controls. In addition, HF feeding had no effect on the percentage alternation or number of moves in Non-Tg or 3xTgAD mice. At 11-12 months of age, the 3xTgAD mice on a chow diet exhibited fewer percentage alternations (19% \( p < 0.05 \); Figure 5.12.C) than the Non-Tg control mice on a chow diet. In addition, an HF diet also impaired memory as the number of percentage alternations was reduced in 11-12-month-old the Non-Tg compared to chow fed controls (26%; \( p < 0.01 \)). However, at 11-12 months of age, an HF diet did not appear to affect memory in 3xTgAD mice as there was no difference in the percentage alternations in HF fed 3xTgAD mice when compared to 3xTgAD mice on Chow. Furthermore, no difference in the total number of moves was observed in any groups regardless of genotype or diet at 11-12 months of age (Figure 5.12.G). At 15-16 months of age 3xTgAD mice on a chow diet performed fewer percentage alternations (19% \( p < 0.05 \); Figure 5.12.D) than the Non-Tg mice on a chow diet. HF affected memory in 15-16-month-old 3xTgAD and Non-Tg mice as these mice performed lower percentage alternations (19%; \( p < 0.05 \) and \( p < 0.01 \)) than their respective
controls on a chow diet. However, genotype or diet had no affect on the number of moves in 15-16-month-old mice (Figure 5.12.H).

**Figure 5.12. Effect of a high fat (HF) diet on Y-maze spontaneous alternation (SA) performance in 3xTgAD mice at 3-4, 7-8, 11-12 and 15-16 months of age**

Between 4-8 weeks of age, cohorts of male triple transgenic 3xTgAD and age-matched Non-Tg mice were fed a chow diet, then at 8 weeks the mice were either kept on chow or placed on an HF diet. At 3-4, 7-8, 11-12 and 15-16 months of age, cohorts (Non-Tg/chow; n= 8-11, Non-Tg/HF; n= 6-12, 3xTgAD/chow; n = 8-10 and 3xTgAD/HF; n=10) were individually placed in a Y-maze and allowed to explore for 8 min. Percentage of alternations (A-D) and total number of moves (E-H) were measured. Data are 24 h means ± SEM. Two-way analysis of variance (ANOVA) with Tukey’s post-hoc analysis, *p < 0.05, **p < 0.01 3xTgAD/chow versus Non-Tg/chow or 3xTgAD/HF versus Non-Tg/HF, #p < 0.05, ##p < 0.01, 3xTgAD/chow versus 3xTgAD/HF or Non-Tg/chow versus Non-Tg/HF.
5.3.2.3. Effect of an HF diet on the Morris water maze (MWM) task in 3xTgAD mice at 3-4, 7-8, 11-12 and 15-16 months of age

Training

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

During submerged platform training, the Non-Tg mice on a chow diet showed significantly decreasing escape latency over training days at all ages tested (days 4-8 versus day 1; p < 0.05 - p < 0.001, Figure 5.13. A-D), whereas the 3xTgAD mice showed no evidence of learning. On an HF diet, the Non-Tg mice got quicker over training days at 3-4, 7-8, 11-12 and 15-16 months of age (days 2-8 versus day 1; p < 0.05 - p < 0.001), whereas the 3xTgAD mice on an HF diet, only got quicker at 3-4 months of age (days 7-8 versus day 1; p < 0.05) yet were unable to learn at 7-8, 11-12 and 15-16 months of age. On individual training days there was no significant difference in escape latency between the cohorts.

Probe test

Two-way ANOVA examining the percentage time in the target quadrant at 3-4 months of age (Figure 5.13.E), revealed an effect of genotype (F_{3,39} = 29.33, p < 0.001) but no effect of diet (F_{3,39} = 0.04, p > 0.05) nor any interaction (F_{3,39} = 0.09, p > 0.05). Two-way ANOVA examining the percentage time in the target quadrant at 7-8 months of age (Figure 5.13.F), revealed an effect of genotype (F_{3,38} = 23.18, p < 0.001), diet (F_{3,38} = 13.2, p < 0.001) but no interaction (F_{3,38} = 0.54, p > 0.05). Two-way ANOVA examining the percentage time in the target quadrant at 11-12 months of age (Figure 5.13.G), revealed an effect of genotype (F_{3,34} = 18.38, p < 0.001) but no effect of diet (F_{3,34} = 0.59, p > 0.05) nor any interaction (F_{3,34} = 0.50, p > 0.05). Two-way ANOVA examining the percentage time in the target quadrant at 15-16 months of age (Figure 5.13.H), revealed an effect of genotype (F_{3,39} = 14.1, p < 0.001) but no effect of diet (F_{3,39} = 0.05, p > 0.05) nor any interaction (F_{3,39} = 0.24, p > 0.05).

Posthoc analysis revealed that during the probe test on a chow diet, the 3xTgAD mice displayed deficits in the percentage time in the target quadrant at 3-4, 7-8 and 15-16 months of age when compared to Non-Tg control mice on a chow diet (38% - 54%; p < 0.05 - p < 0.001, Figure 5.13. E-H). An HF diet worsened memory in the probe test in both the Non-Tg mice and 3xTgAD mice at 7-8 months of age when compared to their respective controls on a chow diet (30% and 32%; p < 0.05, Figure 5.13.F). Similarly, the 3xTgAD mice on an HF diet performed significantly worse than the Non-Tg mice on an HF diet (43%; p < 0.01) at 11-12 months of age, at an age when the 3xTgAD mice on a chow diet did not show a significant memory deficit.
Figure 5.13. Effect of a high fat (HF) diet on the Morris water maze (MWM) task in 3xTgAD mice at 3-4, 7-8, 11-12 and 15-16 months of age

Between 4-8 weeks of age cohorts of male 3xTgAD and Non-Tg mice were fed a chow diet, and then at 8 weeks the mice were either kept on chow or placed on an HF diet. At 3-4, 7-8, 11-12 and 15-16 months of age cohorts (Non-Tg/chow; n = 8-11, Non-Tg/HF; n = 6-12, 3xTgAD/chow; n = 8-10 and 3xTgAD/HF; n = 10) were given four trials a day for 8 d of submerged platform training in the MWM (A-D). Twenty-four hours after the final trial the mice were given a probe test with no platform (E-H). Data are mean +/- SEM. For escape latency, three-way repeated measures analysis of variance (ANOVA) with Scheffe post-hoc analysis. *p < 0.05, **p < 0.01, ***p < 0.001; Non-Tg/chow day 1 versus days 2-8 of training, #p < 0.05, ##p < 0.01, ###p < 0.001; Non-Tg/HF day 1 versus days 2-8 of training, $p < 0.05$ 3xTgAD/HF day 1 versus days 2-8 of training. For percentage time in target, two-way analysis of variance (ANOVA) with Tukey’s post-hoc analysis, *p < 0.05, **p < 0.01 3xTgAD/chow versus Non-Tg/chow or 3xTgAD/HF versus Non-Tg/HF, # p < 0.05 3xTgAD/chow versus 3xTgAD/HF or Non-Tg/chow versus Non-Tg/HF.
5.3.2.4. Effect of an HF diet on memory in the novel object recognition (NOR) test in 3xTgAD mice at 3-4, 7-8, 11-12 and 15-16 months of age

Overall at 3-4, 7-8, 11-12 and 15-16 months of age there was no difference in exploration of identical objects during phase 1 of the test (data not shown). At 3-4 and 7-8 months of age, during phase 2 after an interval of 1h, the Non-Tg mice on a chow or HF diet spent significantly more time exploring the novel versus the familiar object (chow; 42% and 15%; \( p < 0.001 \) and \( p < 0.05 \), HF; 92% and 53%; \( p < 0.001 \), Figure 5.14.A-B). At 11-12 and 15-16 months of age, the Non-Tg mice on a chow diet also spent significantly more time exploring the novel versus the familiar object (95% and 62%; \( p < 0.01 \), Figure 5.14.C-D), whereas an HF diet impaired memory at these ages as HF fed Non-Tg mice spent a similar amount of time exploring both objects. In the 3xTgAD mice, memory was impaired at all ages, as mice on either a chow or HF diet explored both novel and familiar objects to a similar degree.

**Figure 5.14. Effect of a high fat (HF) diet on memory in novel object recognition (NOR) test in 3xTgAD mice in 3-4, 7-8, 11-12 and 15-16 months of age**

Between 4-8 weeks of age cohorts of male triple transgenic 3xTgAD and age-matched Non-Tg mice were fed a chow diet, then at 8 weeks the mice were either kept on chow or placed on an HF diet. At 3-4, 7-8, 11-12 and 15-16 months of age cohorts (Non-Tg/chow; \( n = 8-11 \), Non-Tg/HF; \( n = 6-12 \), 3xTgAD/chow; \( n = 8-10 \) and 3xTgAD/HF; \( n = 10 \) underwent the NOR test. During phase 1 mice were placed in a NOR arena for 10 min with two identical novel objects (Data not shown). During phase 2 (A-D) after an interval of 1h the mice were placed back into the arena with one familiar object (presented in phase 1) and one novel object. Percentage time exploring objects were compared using Student’s t-tests (*\( p < 0.05 \), **\( p < 0.01 \), ***\( p < 0.001 \)).
5.3.2.5. Effect of an HF diet on memory in the odour recognition (OR) test in 3xTgAD mice at 3-4, 7-8, 11-12 and 15-16 months of age

Overall at 3-4, 7-8, 11-12 and 15-16 months of age there was no difference in exploration of identical scented balls during phase 1 (data not shown). During phase 2, after a delay of 3min, the Non-Tg mice at all ages on a chow diet spent a significantly higher percentage of time exploring the novel scented ball than the familiar scented ball previously presented in phase 1 indicating memory formation (21% - 56%, \( p < 0.05 \), Figure 5.15. A-D). Memory was unimpaired in the 3xTgAD mice on a chow diet at earlier time points, as mice spent more time exploring the novel scented ball at 3-4 months of age (108%; \( p < 0.01 \), Figure 5.15. A) and more time exploring the familiar scented ball at 7-8 months of age (118%; \( p < 0.05 \), Figure 5.15. B). However, memory was impaired in chow fed 3xTgAD mice at 11-12 and 15-16 months of age, as no difference in exploration between the novel and familiar smell was observed (Figure 5.15. C-D). HF feeding transiently impaired memory in the Non-Tg mice, as no preference for the exploring of the novel or familiar scented ball was detected at 3-4 and 7-8 months of age (Figure 5.15. A-B), but by 11-12 and 15-16 months of age HF fed Non-Tg mice spent more time exploring the novel scented ball (40% and 35%, \( p < 0.05 \), Figure 5.15. C-D). In contrast, the 3xTgAD mice on an HF diet showed impaired memory at all ages, as they did not distinguish between the novel and familiar smell.
Figure 5.15. Effect of a high fat (HF) diet on memory deficits in odour recognition (OR) test in 3xTgAD mice at 3-4, 7-8, 11-12 and 15-16 months of age

Between 4-8 weeks of age, cohorts of male triple transgenic 3xTgAD and age-matched Non-Tg mice were fed a chow diet, then at 8 weeks the mice were either kept on chow or placed on an HF diet. At 3-4, 7-8, 11-12 and 15-16 months of age cohorts (Non-Tg/chow; n = 8-11, Non-Tg/HF; n = 6-12, 3xTgAD/chow; n = 8-10 and 3xTgAD/HF; n = 10) underwent the OR test. During phase 1, the mice were placed in a SR arena for 10 min with 2 identically scented balls (data not shown). During phase 2 (A-D) after an interval of 3 min the mice were placed back into the arena with one familiar scented ball (presented in phase 1) and one novel scented ball. Percentage time exploring balls were compared using Student’s t-tests (*p < 0.05).
Chapter 5  Effect of a high fat diet on 3xTgAD mice

5.4. Discussion

Both consumption of an HF diet and obesity have been linked with increased incidence of AD. The final aim of this PhD, therefore, was to examine the effect of an HF diet on energy balance, as well as the onset and progression of behaviour and memory deficits in the 3xTgAD mice, compared to Non-Tg control mice. Non-Tg control and 3xTgAD mice displayed similar energy balance profiles in response to an HF diet. Furthermore, an HF diet induced memory impairments, in both Non-Tg and 3xTgAD mice.

5.4.1. Effect of an HF diet on energy balance in 3xTgAD mice

5.4.1.1. Energy balance of control 3xTgAD mice on a chow diet

On a chow diet, the body weight profiles of the Non-Tg control and 3xTgAD mice were similar between cohorts examined during this part of the study and to those seen during the characterisation study (chapter 3), except that aged 3xTgAD mice did not show decreased body weight, when compared to the Non-Tg control mice. However, the overall shape of the body weight profile, in each cohort on a chow diet in this study was the same as that seen in the characterisation study; if the animals would have been monitored for longer in the 15-16 month cohort, then it is possible that the 3xTgAD mice may have started to weigh less than the Non-Tg control mice. Similarly, the increase in metabolic rate was not seen as early as in characterisation study (chapter 3), but body weight was not less at this time point (12 months of age). Furthermore, in the 3xTgAD mice food intake was not significantly increased in this study at any age, when tested using 2-way ANOVA, whereas it was significantly increased at all ages when tested using a Student’s t-test. This discrepancy could be due to the more stringent nature of these analyses, and so future studies may require larger numbers of mice.

No difference in epididymal fat deposition was observed between the 3xTgAD and Non-Tg control mice on chow at all ages. This is of interest, as it suggests that the increase in body weight seen in the 3xTgAD mice at 3-4 months of age is unlikely to be due to an increase in epididymal fat deposition, although it is possible that an increase in subcutaneous and other visceral fat deposits may be responsible. Future studies would need to be done (i.e. DEXA) to evaluate whole body adiposity in more detail. Overall, these results on energy balance are similar to those seen during the characterisation study and previous studies in 3xTgAD mice (Gimenez-Llort et al 2007; Halagappa et al 2007). As these studies were discussed earlier (chapter 3) they will not be mentioned here.
5.4.1.2. Effect of a high fat diet on energy balance in 3xTgAD mice

Both Non-Tg and 3xTgAD mice put on more weight in response to an HF diet than respective controls on a chow diet, although initially the 3xTgAD mice were slower to start gaining weight, possibly due to an increased caloric intake that was observed in the Non-Tg mice in the CLAMS calorimetry cages, which was not seen in the 3xTgAD mice. It must also be noted that the 3xTgAD mice started off heavier than the Non-Tg mice, which may have affected their initial response to being on an HF diet. The overall body weight and caloric profiles of Non-Tg and 3xTgAD mice were similar in response to an HF diet, with both the Non-Tg and 3xTgAD mice consuming significantly more calories on an HF diet than on a chow diet during the period in the CLAMS calorimetry cages, leading to an increased body weight. An HF diet reduced the RQ, as expected for fat oxidation, and appeared to lower the metabolic rate, in both Non-Tg mice and 3xTgAD mice. However, examination of metabolic rate in obese versus lean animals requires the measurement of lean mass (mass excluding fat), as lean tissue mass is the most metabolically active part of the body (Muller et al 2002). The decrease in metabolic rate observed here in HF fed mice, may be due to the fact that body weight, instead of lean mass, is being used in the calculation of VO$_2$ and VCO$_2$. Future studies would be required to measure lean and fat free mass (using DEXA, for example) in order to fully examine metabolic rate in response to an HF diet.

Epididymal fat deposition was increased in the Non-Tg mice on an HF diet, at 3-4 and 7-8 months, which is expected, as they had increased body weight at these ages. However, at 11-12 and 15-16 months of age they no longer had an increased epididymal fat pad weight, despite their increased body weight. The epididymal fat pad is just one fat depot that is normally representative of adipose tissue throughout the body. However, it is possible that there are differences in other fat depots that were not analysed, such as other visceral fat pads or subcutaneous fat, therefore future studies would need to analyse whole body fat. Epididymal fat was increased in the 3xTgAD mice on HF, compared to 3xTgAD mice on a chow diet at all ages, even at 3-4 months of age, when these mice showed no increase in body weight in response to an HF diet. The 3xTgAD mice had lower epididymal fat weight than the Non-Tg control mice on an HF diet at 3-4 months, which was expected, as they were slower to put on weight on the HF diet. At 7-8, 11-12 and 15-16 months of age, the 3xTgAD mice deposited more fat in their epididymal fat pads than the Non-Tg mice on an HF diet, which is of interest, as there was no difference in body weight between groups at these time points. As stated above, epididymal fat is only one fat depot and future studies would need to analyse whole body fat and also assess lean tissue mass. Detailed examination of adiposity in response to an HF diet remains to be done in any model of AD.
5.4.1.3. Previous HF studies in mouse models of AD

To date, no studies have examined in detail the effect of HF feeding on energy balance in AD mouse models. The majority of studies that report an effect on an HF diet on body weight and energy intake in AD mice focus on pathology and none have examined energy expenditure.

Only one study has examined the effect of an HF diet on energy balance in the 3xTgAD model of AD. When fed a 60% HF diet between 4-13 months of age, Non-Tg mice gained more weight compared to respective controls on a chow diet, whereas the body weight of 3xTgAD mice was not affected by the HF diet (Julien et al 2008). The difference in weight gain between the Non-Tg and 3xTgAD on an HF diet appeared to be due to a low animal number and large variability in weight gain in the HF fed 3xTgAD mice. Furthermore, 3xTgAD mice on a chow diet were heavier than Non-Tg mice on a chow diet, yet on an HF diet no difference in 3xTgAD and Non-Tg mice was observed. This is interesting, as we observed that 3xTgAD mice were slower to gain weight in the present study. The authors also noted that on an HF diet, 3xTgAD mice had higher levels of cortical Aβ and tau (Julien et al 2008). In contrast, when 3xTgAD mice were put on a 40% calorie restricted diet at 3 months of age for 11 months, the mice lost weight and Aβ and phosphorylated tau levels were reduced (Halogappa et al 2007). Pathology in the mice from the current study in response to an HF diet is yet to be completed.

Studies in other mouse models of AD demonstrate that when fed an HF or high cholesterol diet AD mice gain weight compared to controls on a chow diet and show increased pathology (George et al 2004; Ho et al 2004; Kohjima et al 2010; Levin-Allerhand et al 2002; Pedrini et al 2009; Refolo et al 2000; Shie et al 2002). AD mouse models fed an HF diet also show higher cholesterol levels (Levin-Allerhand et al 2002; Shie et al 2002) and develop insulin resistance (Ho et al 2004; Kohjima et al 2010). Kohjima et al (2010) examined female APP mice on an HF diet, finding higher body weight, total body fat and insulin resistance after 3 months, when compared to APP mice on a chow diet (Kohjima et al 2010). The HF-fed APP mice had similar caloric intake to APP mice on a chow diet between 1 and 2 months, yet showed higher appetite at 3 months of age, which increased even further at 4 months of age, mirroring the increase in Aβ deposition in response to the HF diet. Kohijima and colleagues also found that levels of brain-derived neurotrophic factor (BDNF), known to play a role in feeding behaviour and satiety, were lower in APP mice on an HF diet. The authors suggest that increased pathology seen in APP mice on an HF diet could be a consequence of the down-regulated expression of BDNF, which in turn could have caused higher caloric intake and insulin resistance (Kohjima et al 2010). The authors also suggest that decreased BDNF levels, which are observed in AD patients (Phillips et al 1991) could be involved in the increased appetite seen in these patients. A full evaluation of neuropeptides involved in feeding behaviour remains to be examined in 3xTgAD mice and could be the focus of future studies. Kohijima and colleagues also reported that calorie restricting HF-fed APP mice (by pair-feeding to
the calorie intake of chow fed APP mice) prevented weight gain and insulin resistance. These findings suggest that increased caloric intake due to an HF diet rather than the diet composition itself is responsible for both the obesity and insulin resistance seen in APP mice.

5.4.1.4. Effect of an HF diet on energy balance summary

This is the first study examining longitudinal analysis of energy balance in response to an HF diet in 3xTgAD mice. The main finding is that overall the metabolic response of 3xTgAD mice to an HF diet is not different to Non-Tg control mice—apart from 3xTgAD mice gain weight and deposit fat slightly slower, but then deposit fat to a greater extent at later time points, despite body weight being no different than Non-Tg mice on an HF diet. Overall, the results observed in this study mirror the few studies that have been done examining the effect of an HF diet in models of AD. The pathology of the mice in this study in response to an HF diet remains to be examined.
5.4.2. Effect of an HF diet on behaviour and memory in 3xTgAD mice

An HF diet was shown to induce further memory deficits in 3xTgAD mice, but HF feeding also affected memory in Non-Tg control mice.

5.4.2.1. Behaviour and memory in control 3xTgAD mice on a chow diet

On a chow diet, the 3xTgAD mice had deficits in the OF at 3-4 and 7-8 months of age, characterised by a reduction in activity, which was no longer seen at 11-12 and 15-16 months of age. The 3xTgAD mice showed memory deficits in the NOR test and MWM probe from 3-4 months of age and in the SA and OR tests from 11-12 months of age. Overall, the 3xTgAD mice showed age-dependent deficits in behaviour and memory, consistent with our characterisation study apart from minor differences in onset of impairments, which may be expected between studies performed at different times using different cohorts of mice. The behaviour and memory of 3xTgAD mice on a chow diet was compared to previous studies in 3xTgAD mice and other models of AD in chapter 4, therefore this will not be discussed here.

5.4.2.2. An HF diet induced memory deficits in both Non-Tg control and 3xTgAD mice

Activity

An HF diet partially inhibited the decrease in activity observed in the 3xTgAD mice in the OF activity/anxiety task and Y-maze SA test, whereas an HF diet had no effect on activity in Non-Tg control mice. As a diet-induced increase in activity and exploration in the OF was only seen in 3xTgAD at one time point, it requires future investigation to determine if it is a real effect and its significance.

Memory

An HF diet affected memory, but the way in which it did this was different depending on the task used.

Y-maze SA test

An HF diet enhanced memory deficits in both the Non-Tg control and 3xTgAD mice, however, it took slightly longer for a significant diet-induced deficit to be seen in the Y-maze SA test in 3xTgAD mice.
An HF diet transiently increased memory deficits in both Non-Tg control and 3xTgAD mice. How and why only transient deficits were observed is unknown, although it could be due to variation between cohorts. Future studies need to be completed with the same groups of animals to monitor the full progression of diet-induced memory deficits.

**NOR test**

No effect of an HF diet could be observed in 3xTgAD mice, as a memory deficit was already present at an early time point. However, an age-dependent memory deficit was observed in Non-Tg mice fed an HF diet.

**OR test**

An HF diet decreased the onset of memory deficit in 3xTgAD mice and affected memory in early ages in Non-Tg mice. As with the MWM task, how and why only transient deficits were seen in Non-Tg mice is unknown and requires further investigation.

Overall, an HF diet made memory worse in both Non-Tg and 3xTgAD mice, however in some tasks it was not possible for the HF diet to have an effect in 3xTgAD mice, as they already had a deficit and further changes would not be possible to detect experimentally. Both hippocampally-dependent (Y-maze, SA and MWM tests) and hippocampally-independent tasks (OR and NOR tests) were worsened by an HF diet. Interestingly though, diet-induced deficits in the MWM occurred much earlier than in the Y-maze SA test for both the 3xTgAD and Non-Tg mice. The reason for this is unknown, but could be due to motivational or anxiety issues related to the MWM making it more sensitive at picking up deficits earlier. Similarly, deficits in the OR test in Non-Tg mice were seen earlier than in the NOR test, even though the delay between trials is much shorter in the OR task than the NOR test. The reason for this is also unknown and would require more investigation in future studies, specifically examining pathology within the brain for any potential diet-related changes.

**5.4.2.3. The effect of an HF diet on behaviour and memory in models of AD**

Very few studies have investigated the effect of an HF diet on behaviour and memory in models of AD. HF feeding increases Aβ and tau levels within the brain of 3xTgAD mice (Julien et al 2008), however the authors did not examine behaviour and memory. Pathology is yet to be examined and correlated to behaviour and memory in our study. More recently, 5-month-old 3xTgAD mice were put on an HF diet for 4 months, showing increased Aβ and tau deposition (Ma et al 2009). 3xTgAD
mice on a HF diet also displayed reduced performance in the Y-maze SA test after 1 and 2 months of HF diet, when compared to Non-Tg control mice on an HF diet. 3xTgAD mice in the study by Ma and colleagues 3xTgAD mice were put on the diet much later than in the present study which may affect onset of cognitive deficits. In the present study deficits on an HF were observed much later, the reasons for this may be due to the extra cholesterol added into this HF diet (1500 ppm versus 269 ppm in the present study). Body weight and energy balance was not described in this study, furthermore, no chow-fed mice were used in this study, however the authors noted that no deterioration in performance was observed between 1 and 2 months of HF diet in Non-Tg mice (Ma et al 2009). It was also not clear what gender the mice were used in this study. In contrast to the effects of excess calorie intake induced by an HF diet, a 40% calorie restricted diet for 7 or 14 months attenuated the reduction in rearing activity within an OF and improved performance in the MWM in 3xTgAD mice (Halagappa et al 2007). These beneficial effects of a calorie restriction on AD-related behavioural deficits in 3xTgAD mice are also associated with a reduction in Aβ and tau levels (Halagappa et al 2007). To date, no studies have investigated the effect of an HF diet on both longitudinal energy balance and a battery of behaviour and memory tasks in 3xTgAD mice. Studies in other models of AD demonstrate that an HF diet can induce memory deficits. After 5 months of HF feeding, female APP mice at 9 months of age show impairments in the submerged platform learning and memory retention in the probe test compared to APP mice on a chow diet (Ho et al 2004). However, no Non-Tg control mice were used in this study. As HF feeding in this PhD caused memory deficits in both 3xTgAD and Non-Tg control mice, the diet-induced deficits seen in models of AD may therefore not be specific to AD, but rather to cognition in general.

5.4.2.4. An HF diet affects memory in cognitively normal rodents and humans

There are a multitude of studies demonstrating that an HF diet worsens memory in cognitively normal rodents (Granholm et al 2008; Greenwood & Winocur 1990; Kanoski & Davidson 2010; McNeilly et al 2010; Murray et al 2009; Valladolid-Acebes et al 2010; Winocur & Greenwood 2005). However, most studies have only assessed spatial memory and have not evaluated the response to an HF diet in a battery of different tests. In the present study, we found that both spatial and non-spatial memory are impaired by an HF diet, in both Non-Tg control and 3xTgAD mice. Interestingly, a recent study showed that spatial reference memory in the radial arm maze is impaired in rats following only 72 h of a 40% HF diet, whereas non-spatial reference memory is only affected after 30 days of HF diet (Kanoski & Davidson 2010). These data suggest that spatial memory is more sensitive to disruption than non-spatial memory. The authors also noted that once a diet-induced deficit was observed, it was stable over time. Similarly, we noted in this study that diet-induced deficits appeared in Non-Tg mice in the Y-maze SA and the NOR test, however we saw transient diet-induced deficits in Non-Tg mice in the MWM probe test and the OR test. However, each time point in the present study was a different cohort of mice and it is possible that the onset of diet-induced deficits could vary between cohorts. In addition to the detrimental effects of HF diets on
cognition in rodents, evidence also suggests that obesity and a poor diet have detrimental effects on cognition in humans (Elias et al. 2003; Jeong et al. 2005). The Framingham heart study reported impaired cognitive function in obese individuals versus those that were not obese (Elias et al. 2003). Similarly, a study of healthy adults (20-82 years) revealed that BMI was inversely related to cognition, with obese participants (BMI > 30) performing poorer than those with a normal weight (BMI = 18.5-24.9) (Gunstad et al. 2007).

5.4.2.5. Potential mechanisms underlying diet-induced memory deficits

The mechanism underlying HF diet induced memory deficits are unknown, but studies in obese humans and animal models suggest that insulin resistance may play an important role. Insulin is thought to play a part in modulating synaptic plasticity and memory, as insulin receptors and insulin-sensitive glucose transporters are expressed in the medial temporal lobe, which is associated with memory (Naderali et al. 2009). Therefore, problems with insulin signalling in the brain could contribute to impaired memory function, as obesity is linked with insulin resistance (Fatani et al. 2007; Lloyd et al. 2009; Naderali & Fatani 2005; Whitaker et al. 1997), and studies have shown that people with insulin resistance have worse cognition (Elias et al. 2003; Gunstad et al. 2007). Studies evaluating HF diet-induced cognitive deficits have demonstrated that rodents fed a HF diet show increased levels of cholesterol, leptin, triglycerides and exhibit insulin resistance (Granholm et al. 2008; McNay et al. 2010; Murray et al. 2009; Valladolid-Acebes et al. 2010; Winocur & Greenwood 2005). HF diet-induced deficits in Y-maze SA are seen in rats that are hyperglycaemic and hyperinsulinemic after 20 weeks of HF diet (McNay et al. 2010). Rats also show a dose-response improvement in spatial working memory following intrahippocampal administration of insulin. Furthermore, blocking endogenous intrahippocampal insulin signalling impaired spatial memory in rats (McNay et al. 2010). It has been hypothesised that insulin resistance and decreased glucose uptake are involved in cognitive deficits on an HF diet (Winocur & Greenwood 2005). Studies have shown that glucose treatment can temporally improve memory in aged rats, potentially by aiding glucose metabolism within the brain. It is possible that the hippocampus is sensitive to insulin resistance, causing impaired glucose regulation and reduced glucose uptake within the hippocampus, which may explain memory deficits observed on an HF diet (Winocur & Greenwood 2005). Furthermore, obesity and HF feeding is associated with changes in many systems such as the upregulation of pro-inflammatory cytokines, which may also be linked to decreased cognition (Bellinger et al. 1995; Gemma & Bickford 2007; Jankowsky & Patterson 1999; Viljoen & Koorts 2004). Studies in rodents have shown that memory deficits in response to an HF diet are associated with brain inflammation (Pistell et al. 2009).

The mechanisms underlying any association between increased adiposity on memory deficits in AD are unknown, although increased body fat (both BMI and fat mass) has been correlated with decreased neuronal viability in areas of the brain associated with learning and memory (Gazdzinski
et al 2008) and increased plasma and brain Aβ levels (Balakrishnan et al 2005). As previously described, obesity itself is associated with insulin resistance and upregulation of proinflammatory cytokines, which may explain the cognitive decline observed in obese humans (Viljoen & Koorts 2004), all of which have been seen in AD (Benveniste et al 2001; Combs 2009; Craft 2005). Increasingly evidence suggests that type II diabetes is associated with higher risk in AD (Ott et al 1999; Schrijvers et al 2010). For this reason, a potential molecular pathway has been proposed to explain the potential increased risk of AD associated with obesity (see Figure 5.16.). This model is based on the role of insulin resistance and hyperinsulinemia on the pathophysiology of AD and neuroinflammation, leading to increased neurodegeneration and cognitive decline (Craft 2005; Neumann et al 2008).

**Figure 5.16. Schematic representation of potential molecular pathways linking obesity and cognitive decline**

Obesity leads to peripheral insulin resistance (a) and hyperinsulinemia (b) which together with a potential contribution of central insulin resistance, leads to increased levels of insulin in the central nervous system (CNS), which reduces the insulin degrading enzyme and thus result in reduced degradation of Alzheimer’s disease (AD), hence upregulating cyclin dependent kinase 5 (Cdk5) leading to tau hyperphosphorylation. Peripheral insulin resistance (c), associated with decreased neuronal insulin signalling, together with decreased phosphoinositol 3,4,5 triphosphase (PI3K) and protein kinase B (PKB), leading to increased glycogen synthase kinase 3 (GSK3β), which is linked to increased tau hyperphosphorylation. Aβ oligomers (d) are thought to activate an innate immune response in glia and lead to neuroinflammation (e), which in turn is associated with neurodegeneration and cognitive decline. PKB downregulation and high GSK3β are associated with pro-apoptotic pathways leading to neurodegeneration (f). Chronic hyperinsulinemia is associated with down-regulation of insulin receptors in the blood brain barrier leading to decreased insulin levels in the CNS (g). Figure redrawn and adapted from (Neumann et al 2008).
Studies in mouse models of AD have demonstrated that consumption of an HF diet is associated with diet-induced memory deficits and/or increased pathology within the brain (George et al 2004; Ho et al 2004; Julien et al 2008; Kohjima et al 2010; Levin-Allerhand et al 2002; Ma et al 2009; Pedrini et al 2009; Refolo et al 2000; Shie et al 2002). In the present study, however, behaviour remains to be correlated to pathology. Studies in single and double transgenic models of AD have shown that an HF diet is associated with insulin resistance (Ho et al 2004; Kohjima et al 2010). Very recently, a study found that 3xTgAD mice with streptozocin-induced diabetes display increased Aβ pathology and that treatment with exendin-4 improved the amyloidogenic effect of diabetes and reduced hyperglycemia. In contrast, no effect on tau was observed (Li et al 2010). After 4 months of an HF diet, 3xTgAD mice displayed increased active c-jun N-terminal kinase (JNK) and phosphorylated insulin receptor substrate-1 (IRS-1) and tau and increased memory deficits (Ma et al 2009). The limited studies in 3xTgAD mice fit in with the proposed model of cognitive decline associated with obesity in AD. The mechanisms underlying memory deficits in Non-Tg control and 3xTgAD mice on a high fat diet in the present study diet are unknown, therefore future studies could assess metabolic parameters, insulin sensitivity and levels of proinflammatory cytokines in response to an HF diet. It remains to be determined if an HF diet causes memory deficits via the same or different mechanisms in both Non-Tg control and 3xTgAD mice, therefore, this is an area of research requiring more investigation. Understanding the mechanism of how an HF diet affects cognition in both the Non-Tg control and 3xTgAD mice may provide therapeutic targets for AD.

5.4.2.6. Effect of an HF diet on energy balance and behaviour and memory summary

AD is a progressive neurodegenerative disorder, characterised by deficits in language, behaviour and memory. Increasing evidence suggests that consuming an HF diet and obesity are risk factors for AD. The data presented in this PhD provide the first longitudinal study to investigate the effect of an HF diet on energy balance and behaviour and memory using a battery of tests, in Non-Tg control and 3xTgAD mice. Non-Tg control and 3xTgAD mice displayed similar energy balance profiles in response to an HF diet. Furthermore, a HF diet induced memory impairments, in both Non-Tg and 3xTgAD mice. As HF feeding also caused memory deficits in Non-Tg mice, the diet-induced impairment may therefore not be specific to AD, but rather to memory overall. These results stress the need for control groups of Non-Tg mice fed HF diets, to determine whether any effects of diet observed in AD mice are specific to the disease itself or cognition in general. The mechanisms involved in the dietary effect on memory remain poorly understood.
Chapter 6

Final discussion
6.1 Final discussion

AD is a progressive neurodegenerative disorder, characterised by deficits in language, behaviour and memory. Increasing evidence suggests that mid-life obesity and a diet high in fat are risk factors for AD (Fitzpatrick et al 2009; Rosengren et al 2005; Whitmer et al 2005; Whitmer et al 2008). In contrast, life-threatening weight loss occurs and worsens as the disease progresses (Gillette Guyonnet et al 2007; Gillette-Guyonnet et al 2000; Guerin et al 2005; White et al 1996), despite adequate or increased food intake (Burns et al 1989; Keene & Hope 1997a; b; Niskanen et al 1993; Singh et al 1988; Smith et al 1999; Spindler et al 1996). A greater understanding of energy balance in AD may, therefore, uncover novel targets for therapy. The aim of my PhD was to further study how energy balance might be involved in AD, using an experimental model of the disease. Specific objectives were to:

- characterise the energy balance profile of 3xTgAD mice
- characterise behaviour and memory in a battery of tests in 3xTgAD mice
- evaluate the effect of an HF diet on cognition in 3xTgAD mice

Firstly, energy balance was characterised in Non-Tg control and 3xTgAD mice (see summary Table 6.1.), demonstrating altered body weight, food intake and metabolic rate in 3xTgAD mice. At 2-month of age male 3xTgAD mice displayed greater food intake and body weight, but no difference in metabolic rate, whereas from 12 months of age 3xTgAD mice weighed less, despite eating more, and had a higher metabolic rate than Non-Tg control mice. This provides evidence that there is a shift towards a hypermetabolic state from 12 months of age in 3xTgAD mice, which may represent a key stage in advancement of the disease process. This work is completely novel, as it is the first longitudinal evaluation of energy balance including assessment of metabolic rate reported in any model of AD. As changes in energy balance are reported throughout the progression of AD, the 3xTgAD model represents a useful tool to better understand changes in energy balance and underlying mechanisms.

| Table 6.1. Summary of the characterisation of energy balance in 3xTgAD mice versus Non-Tg control mice increase (↑, increase; ↓, decrease and ↔, no difference) |
|-----------------|-----------------|-----------------|-----------------|
| Age             | Body weight     | Food intake     | Metabolic rate  |
| 2 months        | ↑               | ↑               | ↔               |
| 12 months       | ↓               | ↑               | ↑               |
| 18 months       | ↓               | ↑               | ↑               |

132
Secondly, behaviour and memory were characterised in Non-Tg control and 3xTgAD mice in a battery of tests at different ages (see summary Table 6.2.). 3xTgAD mice showed changes in OF activity/anxiety from 3 months of age. Memory impairments were first detected in 3xTgAD mice at 3 months of age as deficits in OR, mirroring early impairments in odour memory seen in AD patients. Deficits in spatial memory were then observed in both the Y-maze SA and MWM tasks from 5 months of age. Finally, deficits in non-spatial visual object memory were observed in 3xTgAD mice in the NOR at 8 months of age. Overall, 3xTgAD mice displayed age-dependent memory deficits consistent with previous studies.

Table 6.2. Summary of the characterisation of behaviour and memory in 3xTgAD mice versus Non-Tg control mice (m, months; x, no difference; ✓ change in OF behaviour or memory deficit in all other tests; - not tested at this age; OR, odour recognition; OF, open-field; SA, spontaneous alternation; MWM, Morris water maze and NOR, novel object recognition)

<table>
<thead>
<tr>
<th>Test</th>
<th>OR</th>
<th>OF</th>
<th>SA</th>
<th>MWM</th>
<th>NOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>3 months</td>
<td>5 months</td>
<td>8 months</td>
<td>12 months</td>
<td></td>
</tr>
<tr>
<td></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>✓</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>x</td>
<td>✓</td>
<td>-</td>
<td>✓</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>3 months</td>
<td>5 months</td>
<td>8 months</td>
<td>12 months</td>
<td></td>
</tr>
<tr>
<td></td>
<td>OR</td>
<td>✓</td>
<td>✓</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td>OF</td>
<td>✓</td>
<td>✓</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td>SA</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td>MWM</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td>NOR</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
</tbody>
</table>

The final part of the thesis went on to assess energy balance by disrupting it experimentally. This was done by putting Non-Tg control and 3xTgAD mice on a chow or HF diet and assessing energy balance, behaviour and memory at 3-4, 7-8, 11-12 or 15-16 months of age (see summary Table 6.3.).

Table 6.3. Summary of the effect of a high fat (HF) diet on a battery of cognitive tests in 3xTgAD and Non-Tg control mice versus respective controls on a chow diet (m, months; x, no difference; ✓ change in OF behaviour or memory deficit in all other tests; OR, odour recognition; MWM, Morris water maze; OF, open-field; SA, spontaneous alternation and NOR, novel object recognition)

<table>
<thead>
<tr>
<th>Test</th>
<th>Non-Tg</th>
<th>3xTgAD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>3-4 m</td>
<td>7-8m</td>
</tr>
<tr>
<td>OR</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>MWM</td>
<td>x</td>
<td>✓</td>
</tr>
<tr>
<td>OF</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>SA</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>NOR</td>
<td>x</td>
<td>x</td>
</tr>
</tbody>
</table>
Non-Tg control and 3xTgAD mice displayed similar energy balance profiles in response to an HF diet. The HF diet was found to worsen memory in Non-Tg mice in OR at 3-4 and 7-8 months of age, in the MWM at 7-8 months of age and in NOR and SA at 11-12 and 15-16 months of age. Similarly, the HF diet worsened memory in 3xTgAD mice in OR at 3-4 and 7-8 months of age, the MWM at 7-8 and 11-12 months of age, and in SA at 15-16 months of age. As an HF diet induced memory impairments, in both Non-Tg control and 3xTgAD mice, it suggests that diet-induced deficits may therefore, not be specific to AD, but rather to cognition in general. These data support an important role for energy balance in the progression of AD as well as normal ageing. This work is completely novel as this is the first HF study in both Non-Tg control and 3xTgAD mice with longitudinal evaluation of energy balance with a battery of different behavioural tests.

In AD, much remains unknown in the area of energy balance, as we do not know exactly when, how or why changes in energy balance occur. For this reason, energy balance needs to be examined in more detail, assessing caloric intake, adiposity and metabolic rate longitudinally in a more homogenous AD population than used to date, together with appropriate age-matched nondemented controls. However, as previously described, changes in energy balance may occur years before patients go to a clinic. Ideally, it would be great to assess energy balance throughout life in a cohort of humans and see what diseases they get along the way. However, realistically this is almost impossible, even more so if investigating risk factors, such as diet. Unlike humans, mice have a shorter life span, are kept in a controlled environment and can be fed identical diets. This makes understanding diseases, such as AD, and associated risk factors much easier, as you have a homogenous cohort of animals, which you can study in great detail. As 3xTgAD mice display alterations in energy balance throughout life and display cognitive deficits in response to an HF diet, they represent an appropriate model.

Future studies need to determine the exact timing of changes in body weight and metabolic rate on a chow diet in more detail and correlate them with both behaviour and pathology. It would also be interesting to try and prevent the lower body weight seen in aged male 3xTgAD mice on a chow diet, compared to Non-Tg control mice and see how that affects their energy balance as well as progression of pathology and cognitive decline. Feeding the 3xTgAD mice a higher calorific diet would be one way to do this. However, this would be difficult as would need to measure intake very carefully to prevent them weighing more than the Non-Tg control mice. A better way would be to fully understand why the 3xTgAD mice weigh less and an intervention study based on this.

How an HF diet affects cognition in 3xTgAD mice also requires further work as different tasks were affected at different time points. However, it is difficult to compare time points in the present study, as they are in different cohorts of mice. Ultimately, cohorts of 3xTgAD and Non-Tg controls on a chow or HF diets would have cognition assessed at different time points throughout their lives with pathology being assessed with fMRI imaging techniques. In the present study, the effect of diet on pathology remains to be completed in 3xTgAD and correlated with cognition. Interestingly, it
also appears that HF diets can induce cognitive decline independently of AD phenotype, stressing the need for control groups of HF-fed Non-Tg mice in HF studies in transgenetic models. The mechanisms underlying HF diet-induced deficits in both Non-Tg control and 3xTgAD mice require further examination. This could be done by putting cohorts of Non-Tg and 3xTgAD mice on the same HF diet used, during this thesis, and comparing their cognition with other cohorts on the same HF diet, but varying in one component such as increasing the level of cholesterol or changing the fatty acid ratio within the diet. It would also be interesting to put 3xTgAD mice on a HF diet and then try various intervention studies (low fat diet/caloric restriction, exercise sessions) to see how they affect progression of cognitive decline and pathology. As insulin resistance may be implicated in the effect of obesity on cognition, insulin resistance should be assessed in Non-Tg control and 3xTgAD mice, used in this thesis. In future studies it would then be interesting to try anti-diabetes treatments, such as Metformin, and see how cognition and pathology are affected.

Overall, there is an urgent need to identify disease-modifying treatments for AD, and data presented here indicate that energy balance could represent an attractive target. However, further clinical studies are needed to fully establish the profile of body weight and metabolic changes in patients at various stages of the disease. Experimental studies also need to better define the timing of body weight and metabolic changes, and how these correlate with both behaviour and pathology. More detailed analyses of risk factors, such as an HF diet is also required in both clinical and experimental studies to fully understand how they affect AD. Only once this work has been done can targeted interventions be used to determine if they modify progression or severity of the AD-like changes in models of AD, and whether these might be translatable to the clinic.
Appendices
Appendix A: Nutritional profiles of chow and HF diets

Chow diet

BEEKAY RAT AND MOUSE DIET No 1 (BK001E)

This diet is designed for breeding and growth of rodents. It was reformulated in 2002 to remove animal protein (fish meal) and so contains only protein of plant origin. Although a new formulation to the UK it has been tried and tested for several years in Scandinavia for breeding rodents.

It is also an excellent all-purpose diet for maintenance of experimental animals on short or medium duration (less than one year) experiments.

<table>
<thead>
<tr>
<th>Product Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Order Name:</td>
</tr>
<tr>
<td>Main Ingredients:</td>
</tr>
<tr>
<td>Form:</td>
</tr>
<tr>
<td>Presentation:</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Irradiation:</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Calculated Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude Oil %</td>
</tr>
<tr>
<td>4.13</td>
</tr>
<tr>
<td>Crude Protein %</td>
</tr>
<tr>
<td>19.67</td>
</tr>
<tr>
<td>Crude Fibre %</td>
</tr>
<tr>
<td>3.22</td>
</tr>
<tr>
<td>Ash %</td>
</tr>
<tr>
<td>4.71</td>
</tr>
<tr>
<td>NFE %</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Dig Crude Oil %</td>
</tr>
<tr>
<td>3.76</td>
</tr>
<tr>
<td>Dig Crude Protein %</td>
</tr>
<tr>
<td>17.37</td>
</tr>
<tr>
<td>Gross Energy MJ/kg</td>
</tr>
<tr>
<td>16.39</td>
</tr>
<tr>
<td>Dig Energy MJ/kg</td>
</tr>
<tr>
<td>13.75</td>
</tr>
<tr>
<td>Vitamin A iu/kg</td>
</tr>
<tr>
<td>12590</td>
</tr>
<tr>
<td>Vitamin D$_2$ iu/kg</td>
</tr>
<tr>
<td>1574</td>
</tr>
<tr>
<td>Vitamin E mg/kg</td>
</tr>
<tr>
<td>103.4</td>
</tr>
<tr>
<td>Thiamin mg/kg</td>
</tr>
<tr>
<td>13.1</td>
</tr>
<tr>
<td>Riboflavin mg/kg</td>
</tr>
<tr>
<td>11.7</td>
</tr>
<tr>
<td>Pyridoxine mg/kg</td>
</tr>
<tr>
<td>14.1</td>
</tr>
<tr>
<td>Vitamin B12 µg/kg</td>
</tr>
<tr>
<td>20.0</td>
</tr>
<tr>
<td>Vitamin K mg/kg</td>
</tr>
<tr>
<td>15.9</td>
</tr>
<tr>
<td>Folic Acid mg/kg</td>
</tr>
<tr>
<td>3.1</td>
</tr>
<tr>
<td>Nicotinic Acid mg/kg</td>
</tr>
<tr>
<td>73.1</td>
</tr>
<tr>
<td>Pantothenic Acid mg/kg</td>
</tr>
<tr>
<td>26.6</td>
</tr>
<tr>
<td>Choline mg/kg</td>
</tr>
<tr>
<td>1406.9</td>
</tr>
<tr>
<td>Inositol mg/kg</td>
</tr>
<tr>
<td>1811.4</td>
</tr>
<tr>
<td>Biotin µg/kg</td>
</tr>
<tr>
<td>369.2</td>
</tr>
<tr>
<td>Calcium %</td>
</tr>
<tr>
<td>0.84</td>
</tr>
<tr>
<td>Total Phosphorus %</td>
</tr>
<tr>
<td>0.66</td>
</tr>
<tr>
<td>Available Phosphorus %</td>
</tr>
<tr>
<td>0.39</td>
</tr>
<tr>
<td>Magnesium %</td>
</tr>
<tr>
<td>0.17</td>
</tr>
<tr>
<td>Sodium %</td>
</tr>
<tr>
<td>0.18</td>
</tr>
<tr>
<td>Chloride %</td>
</tr>
<tr>
<td>0.31</td>
</tr>
<tr>
<td>Potassium %</td>
</tr>
<tr>
<td>0.85</td>
</tr>
<tr>
<td>Iron mg/kg</td>
</tr>
<tr>
<td>118.4</td>
</tr>
<tr>
<td>Copper mg/kg</td>
</tr>
<tr>
<td>19.2</td>
</tr>
<tr>
<td>Manganese mg/kg</td>
</tr>
<tr>
<td>94.7</td>
</tr>
<tr>
<td>Zinc mg/kg</td>
</tr>
<tr>
<td>92.1</td>
</tr>
<tr>
<td>Cobalt µg/kg</td>
</tr>
<tr>
<td>557.3</td>
</tr>
<tr>
<td>Iodine µg/kg</td>
</tr>
<tr>
<td>1522.7</td>
</tr>
<tr>
<td>Selenium µg/kg</td>
</tr>
<tr>
<td>297.3</td>
</tr>
<tr>
<td>Lysine %</td>
</tr>
<tr>
<td>1.09</td>
</tr>
<tr>
<td>Methionine %</td>
</tr>
<tr>
<td>0.30</td>
</tr>
<tr>
<td>Linoleic Acid %</td>
</tr>
<tr>
<td>1.28</td>
</tr>
<tr>
<td>Linolenic Acid %</td>
</tr>
<tr>
<td>0.16</td>
</tr>
</tbody>
</table>

Labelling

Each bag is labelled with

- Product reference
- Expiry date
- Batch identification

Quality Control Analysis

Independent laboratories perform physical-chemical and microbiological analyses on each batch of diet. Periodic full chemical analyses are conducted on selected batches to assure consistency over time.

Sep 2002
### Basal Purified Diet w/60% Energy From Fat, Blue

**Description**

Basal Purified Diet w/60% Energy From Fat. Dried Blue is based on TestDiet® Basal Diet 5755.

Storage conditions are particularly critical to TestDiet® products, due to the presence of antioxidants or preservative agents. To provide maximum protection against possible changes during storage, store in a dry, cool location. Storage under refrigeration (2-4 °C) is required. If long-term studies are involved, store the diet at -20 °C or colder. Be certain to keep in air tight containers.

**Product Forms Available**

<table>
<thead>
<tr>
<th>Product Form</th>
<th>Catalog #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meal</td>
<td>1810740</td>
</tr>
<tr>
<td>Meal, Irradiated</td>
<td>1810741</td>
</tr>
<tr>
<td>1/2&quot; Pellet</td>
<td>58167</td>
</tr>
<tr>
<td>1/2&quot; Pellet, Irradiated</td>
<td>58170</td>
</tr>
</tbody>
</table>

*Other Forms Available By Request*

### Ingredients

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Catalog #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lard</td>
<td>28.3150</td>
</tr>
<tr>
<td>Casein - Free</td>
<td>26.7600</td>
</tr>
<tr>
<td>Dextrin</td>
<td>18.9872</td>
</tr>
<tr>
<td>Sucrose</td>
<td>6.3715</td>
</tr>
<tr>
<td>RP Min. Mix #10</td>
<td>6.3715</td>
</tr>
<tr>
<td>Corn Oil</td>
<td>6.3715</td>
</tr>
<tr>
<td>RP Vit. Mix (adds 1.94% sucrose)</td>
<td>2.5486</td>
</tr>
<tr>
<td>Powdered Cellulose</td>
<td>1.9114</td>
</tr>
<tr>
<td>Inulin</td>
<td>1.9114</td>
</tr>
<tr>
<td>Choline Chloride</td>
<td>0.2548</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>0.1911</td>
</tr>
<tr>
<td>Blue Dye</td>
<td>0.0060</td>
</tr>
</tbody>
</table>

### Part of the TestDiet® DIO Series

Basal Purified Diet w/12% Energy From Fat

<table>
<thead>
<tr>
<th>Diet Type</th>
<th>Catalog #</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/2&quot; Pellet</td>
<td>581145 (58G7)</td>
</tr>
<tr>
<td>Meal</td>
<td>1810736 (58G7)</td>
</tr>
<tr>
<td>Meal, Irradiated</td>
<td>1810737 (58G7)</td>
</tr>
</tbody>
</table>

Basal Purified Diet w/45% Energy From Fat

<table>
<thead>
<tr>
<th>Diet Type</th>
<th>Catalog #</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/2&quot; Pellet</td>
<td>581146 (58G6)</td>
</tr>
<tr>
<td>Meal</td>
<td>581168 (58G6)</td>
</tr>
</tbody>
</table>

Basal Purified Diet w/70% Energy From Fat

<table>
<thead>
<tr>
<th>Diet Type</th>
<th>Catalog #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meal</td>
<td>58171 (58H0)</td>
</tr>
</tbody>
</table>

### Feeding Directions

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

### CAUTION

Perishable, refrigerate upon receipt.

For experimental use only.

6/28/2005

### Nutritional Profile

<table>
<thead>
<tr>
<th>Protein, %</th>
<th>24.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine, %</td>
<td>0.94</td>
</tr>
<tr>
<td>Histidine, %</td>
<td>0.69</td>
</tr>
<tr>
<td>Isoleucine, %</td>
<td>1.28</td>
</tr>
<tr>
<td>Leucine, %</td>
<td>2.31</td>
</tr>
<tr>
<td>Lysine, %</td>
<td>1.94</td>
</tr>
<tr>
<td>Methionine, %</td>
<td>0.88</td>
</tr>
<tr>
<td>Cysteine, %</td>
<td>0.10</td>
</tr>
<tr>
<td>Phenylalanine, %</td>
<td>1.28</td>
</tr>
<tr>
<td>Tyrosine, %</td>
<td>1.35</td>
</tr>
<tr>
<td>Threonine, %</td>
<td>1.03</td>
</tr>
<tr>
<td>Tryptophan, %</td>
<td>0.30</td>
</tr>
<tr>
<td>Valine, %</td>
<td>1.53</td>
</tr>
<tr>
<td>Alanine, %</td>
<td>0.74</td>
</tr>
<tr>
<td>Aspartic Acid, %</td>
<td>1.72</td>
</tr>
<tr>
<td>Glutamic Acid, %</td>
<td>5.46</td>
</tr>
<tr>
<td>Glycine, %</td>
<td>0.52</td>
</tr>
<tr>
<td>Proline, %</td>
<td>3.15</td>
</tr>
<tr>
<td>Serine, %</td>
<td>1.48</td>
</tr>
<tr>
<td>Taurine, %</td>
<td>0.00</td>
</tr>
</tbody>
</table>

### Minerals

<table>
<thead>
<tr>
<th>Element</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>0.77</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>0.72</td>
</tr>
<tr>
<td>Potassium</td>
<td>0.51</td>
</tr>
<tr>
<td>Magnesium</td>
<td>0.09</td>
</tr>
<tr>
<td>Sodium</td>
<td>0.27</td>
</tr>
<tr>
<td>Chlorine</td>
<td>0.30</td>
</tr>
<tr>
<td>Fluorine</td>
<td>6.3</td>
</tr>
<tr>
<td>Iron</td>
<td>80</td>
</tr>
<tr>
<td>Zinc</td>
<td>35</td>
</tr>
<tr>
<td>Manganese</td>
<td>83</td>
</tr>
<tr>
<td>Copper</td>
<td>19.1</td>
</tr>
<tr>
<td>Cobalt</td>
<td>4.1</td>
</tr>
<tr>
<td>Iodine</td>
<td>0.73</td>
</tr>
<tr>
<td>Chromium</td>
<td>3.9</td>
</tr>
<tr>
<td>Molybdenum</td>
<td>1.04</td>
</tr>
<tr>
<td>Selenium</td>
<td>0.29</td>
</tr>
</tbody>
</table>

### Vitamins

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A, IU/g</td>
<td>28.2</td>
</tr>
<tr>
<td>Vitamin D-3 (added), IU/g</td>
<td>2.8</td>
</tr>
<tr>
<td>Vitamin E, IU/kg</td>
<td>63.8</td>
</tr>
<tr>
<td>Vitamin K (as menadione), ppm</td>
<td>13.25</td>
</tr>
<tr>
<td>Thiamin Hydrochloride, ppm</td>
<td>26.3</td>
</tr>
<tr>
<td>Riboflavin, ppm</td>
<td>25.5</td>
</tr>
<tr>
<td>Niacin, ppm</td>
<td>115</td>
</tr>
<tr>
<td>Pantothenic Acid, ppm</td>
<td>70</td>
</tr>
<tr>
<td>Folic Acid, ppm</td>
<td>5.1</td>
</tr>
<tr>
<td>Pyridoxine, ppm</td>
<td>21.0</td>
</tr>
<tr>
<td>Biotin, ppm</td>
<td>0.5</td>
</tr>
<tr>
<td>Vitamin B-12, mcg/kg</td>
<td>25</td>
</tr>
<tr>
<td>Choline Chloride, ppm</td>
<td>1,784</td>
</tr>
<tr>
<td>Ascorbic Acid, ppm</td>
<td>0.0</td>
</tr>
</tbody>
</table>

### Energy (kcal/g)

<table>
<thead>
<tr>
<th>From</th>
<th>kcal</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>0.969</td>
<td>18.6</td>
</tr>
<tr>
<td>Fat (ether extract)</td>
<td>3.122</td>
<td>59.9</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>1.113</td>
<td>21.4</td>
</tr>
</tbody>
</table>

1. Based on the latest ingredient analysis information. Since nutrient composition of natural ingredients varies, analysis will differ accordingly. Nutrients expressed as percent of ration on an As Fed basis except where otherwise indicated.
2. Energy (kcal/g) - Sum of decimal fractions of protein, fat and carbohydrate x 4,9.4 kcal/gm respectively.
Appendix B: Genotyping of 3xTgAD and Non-Tg control mice

To verify the genotype of 3xTgAD and Non-Tg mice tail snips were genotyped.

To obtain DNA, 0.5 cm of tail was placed in 500 μl of lysis buffer (stock prepared as follows; 2.5 ml of 100 mM Tri-HCl pH 8.5, 250 μl of 5 mM EDTA pH 8, 500 μl of 0.2% SDS, 1 ml of 200 mM NaCl and 250 μl of 100 μg/ml Proteinase K and 20.5 ml sterile water) and placed on a rotor (Labnet, Problot hybridization oven, Edison, NJ, USA) overnight at 55°C. Samples were then spun at 10,000 rpm for 10 min at room temperature (Mikro 200R Hettich zentrifugen, Wolf Laboratories, UK). The tissue lysate was then collected and diluted (20 μl lysate and 90 μl ultra-sterile H2O) and 3 μl DNA lysate added to 47 μl PCR reaction (37 μl sterile water, 5 μl 10x PCR buffer, 3 μl MgCl2, 0.5 μl dNTP, 1 μl primer mix, 0.5 μl Tag DNA polymerase). PS-1 PCR product was digested for 1 h at 60°C with BstE II (Lambda DNA/BstE II digest, Sigma-Aldrich, MO, USA). Negative controls were also prepared by adding distilled water to the PCR reaction instead of DNA lysate). The PCR reaction was then run (Gene AMP 9700 PE Applied Biosystems, Warrington, Cheshire, UK).

PCR programs and primers:

**PS-1 PCR**

5’-CAC ACG CAA CTC TGA CAT GCA CAG GC -3’
5’-AGG CAG GAA GAT CAC GTG TTC AAG TAC -3’

<table>
<thead>
<tr>
<th>Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial denaturation 2.5 min at 94°C</td>
</tr>
<tr>
<td>Denaturation 40 s at 94°C</td>
</tr>
<tr>
<td>Annealing 40 s at 62°C</td>
</tr>
<tr>
<td>Extension 1 min at 72°C</td>
</tr>
<tr>
<td>(35 cycles)</td>
</tr>
<tr>
<td>Final extension 2 min at 72°C</td>
</tr>
<tr>
<td>Hold at 4°C</td>
</tr>
</tbody>
</table>

**APP PCR**

5’-GGT GAG TTT GTA AGT GAT GCC -3’
5’-TCT TCT TCT TCC ACC TCA GC -3’

5’-CAA ATG TTG CTT GTC TGG TG – 3’
5’-GTC AGT CGA GTG CAC AGT TT -3’  Control primer pair
**Conditions**

Initial denaturation 1.5 min at 94°C
---
Denaturation 30 s at 94°C
Annealing 45 s at 60°C
Extension 45 s at 72°C
(35 cycles)
---
Final extension 2 min at 72°C
Hold at 4°C

**TAU PCR**

5'– TGA ACC AGG ATG GCT GAG -3'
5'– TTG TCA TCG CTT CCA GTC C -3'

5'– CAA ATG TTG CTT GTC TGG TG -3'
5'– GTC AGT CGA GTG CAC AGT TT -3'

Control primer pair

**Conditions**

Initial denaturation 5 min at 94°C
---
Denaturation 30 s at 94°C
Annealing 30 s at 60°C touching down by -0.5°C each cycle
Extension 45 s at 72°C
(22 cycles)
---
Denaturation 30 s at 94°C
Annealing 30 s at 50°C
Extension 45 s at 72°C
(13 cycles)
---
Final extension 10 mins at 72°C
Hold at 4°C

PCR products were kept at 4°C until the PCR gel was run. 1.3% agarose in 1xTAE (tris acetic acid EDTA) was made by adding 1.3 g agarose to 100 ml TAE. The solution was then gently microwaved for 3 min and left to cool. Ethidium bromide (5 µl in 100 µl) was then added and the gel was poured and left to set. 15 µl of sample or control and 3 µl DNA loading buffer (5x) were then pipetted on nescofilm. The first lane was loaded with 8 µl of DNA marker (Hyperladder 4, Bioline, London, UK) and 8 µl of the tail snip PCR sample or control and loading buffer were loaded into the other lanes. The gel was run a 100 v for 1 h (Hybaid Electrophoresis tank, Thermoscientific).

All mice used during this PhD were genotyped. For example results see Figure B1.
Figure B1. Genotyping 3xTgAD and Non-Tg control mice

All mice used throughout this PhD were genotyped, A. APP (control primer; mouse DNA is an internal control), B. PS-1 and C. Tau (control primer; mouse DNA as an internal control).
Appendix C: Published manuscript
Hypermethabolism in a triple-transgenic mouse model of Alzheimer’s disease

Elyss M. Knight, Alexei Verkhratsky, Simon M. Luckman, Stuart M. Allan, Catherine B. Lawrence*

Faculty of Life Sciences, University of Manchester, Manchester, M13 9PT, United Kingdom
Received November 25, 2008; received in revised form January 8, 2010; accepted February 9, 2010

Abstract

A common feature of Alzheimer’s disease (AD) is weight loss, even though there is often an increase in food intake in AD patients. The reasons for this weight loss are unknown, but may be due to increased energy expenditure (metabolic rate) or a reduction in energy intake. This was investigated in the present study, using a triple-transgenic (3xTgAD) mouse model of AD. Two-month-old 3xTgAD mice displayed greater food intake (17%) and body weight (34%) but no difference in metabolic rate, as compared with nontransgenic controls (non-Tg). At 12 months of age, 3xTgAD mice still consumed more food (30%), but their body weight was significantly lower (15%) than non-Tg controls. This reduction in body weight was accompanied by a significant rise in metabolic rate, indicated by greater oxygen consumption (24%) and carbon dioxide production (29%); the effects were also observed in 18-month-old 3xTgAD mice. These data demonstrate for the first time the existence of a hypermetabolic state in an experimental model of AD, but whether this can explain the weight loss observed in AD patients remains to be determined.

Keywords: Alzheimer’s disease; Energy balance; Energy expenditure; Hypermethabolism; 3xTgAD; Body weight; Food intake

1. Introduction

Alzheimer’s disease (AD) is an age-related neurodegenerative disorder characterized by a progressive decline in cognitive function that is associated with the presence of amyloid-beta (AB) plaques and neurofibrillary tangles. Although obesity in midlife has been identified as a risk-factor for AD, weight loss is a common feature of the disease, affecting 30%–40% of patients with mild to moderate AD (Gillette et al., 2007; Gillette-Guyonnet et al., 2000; Guerin et al., 2005; White et al., 1996). This weight loss leads to poorer health outcomes, such as an increased risk of infections, falls and muscular atrophy, with a resultant reduction in quality of life and an increase in morbidity. The risk of weight loss increases with the severity and progression of AD, and is a predictor of mortality (White et al., 1998, 2004). Prevention of this life-threatening weight loss in AD is therefore a major goal, but currently the mechanisms responsible are unknown. To lose lean or fat mass, energy intake must be lower than demand (expenditure and storage). However, food intake in people with AD is usually adequate or even increased (Burns et al., 1989; Keene and Hope, 1997a; b; Niskanen et al., 1993; Smith et al., 1999; Spindler et al., 1996).

Weight loss can occur if energy expenditure (metabolic rate) is raised. It is uncertain whether abnormalities in metabolism exist in AD patients because no change, an increase, or a reduction in metabolism has been reported (Donaldson et al., 1996; Pochlman et al., 1997; Niskanen et al., 1993; Prentice et al., 1989; Wang et al., 1997; Wolfs-Klein et al., 1995). Although no direct measurements of metabolic rate in animal models of AD have been reported to date, the existence of a hypermetabolic state is supported...
indirectly by the finding that energy intake is elevated, but weight was lower, in some transgenic mouse AD strains (Pugh et al., 2007; Vloeberghs et al., 2008). Therefore, the aim of this study was to examine longitudinally body weight, food intake, and metabolic rate in a triple-transgenic (3xTgAD) mouse model of AD. These mice overexpress human amyloid precursor protein (APP695), presenilin-1 (PS-1M146V) and tauP301L, and develop progressive age-dependent cognitive deficits as well as Aβ plaque pathology and neurofibrillary tangles (Oddo et al., 2003).

2. Methods

2.1. Procedure

3xTgAD and background strain, wild-type nontransgenic mice (non-Tg) (C57BL/6J29ov), were originally supplied by Frank LaFerla and Salvatore Oddo (University of California, Irvine, CA, USA), and in-house colonies were established as separate litters.

Male mice at 4–5 weeks of age were group-housed in standard housing conditions (temperature, 20°C ± 2°C; humidity, 55% ± 5%; 12-hour light/12-hour dark cycle with lights on at 8:00 hours), and given ad libitum access to standard rodent chow and water. All studies were conducted in accordance with the UK Animals (Scientific Procedures) Act, 1986. Separate groups of 3xTgAD and non-Tg mice had body weight and food intake measured weekly until 2 (n = 4 per group) and 12 months of age (n = 5 for 3xTgAD and n = 6 for non-Tg). At these time points, mice were housed individually in calorimetric cages (Columbus Instruments, Columbus, OH, USA) to allow for measurements of metabolic rate. After 24-hour habituation, oxygen consumption (VO2, in mL/kg/h), carbon dioxide production (VCO2, in mL/kg/h), and respiratory quotient (RQ; ratio of VCO2 to VO2, that is used as an indicator of composition of metabolic fuel being oxidized for energy) were measured by indirect calorimetry every 10 minutes for 4 days. Body weight and food intake were also measured daily and a 24-hour average was calculated. Mean heat production (kcal/d) was calculated and used as a measure of energy expenditure. Mean energy balance over the 4-day monitoring period was calculated from the difference between energy intake (kcal/d) and energy expenditure and expressed as average energy gained or lost per day. A separate group of non-Tg and 3xTgAD mice at 18 months of age were analyzed for body weight, food intake, and metabolic rate over 4 days in calorimetry cages as mentioned earlier (n = 4 per group).

To confirm AD pathological changes, immunohistochemistry for Aβ and hyperphosphorylated tau was performed using 6E10 and AT8 antibodies, respectively, on brain sections from all mice.

2.2. Statistical analyses

Data are represented as mean ± standard error of the mean. For group-housed animals, individual body weight was measured, but average weekly food intake was calculated by dividing weekly food consumption per group by the number of mice per group. Statistical differences in longitudinal body weight (in group-housed animals) were determined using repeated measures analysis of variance. Statistical differences in mean body weight, food intake, VO2, VCO2, RQ, and energy balance over the 4-day monitoring period in calorimetric cages were analyzed using a Student’s t-test. Statistical significance was taken when P < .05.

3. Results

3.1. Food intake, body weight, and metabolic rate in 2-month-old 3xTgAD mice

Between 6 and 8 weeks of age, body weight of 3xTgAD mice was significantly higher by 27%–31% compared with that in age-matched non-Tg control mice (P < .05, Fig. 1A). Average weekly food intake per mouse between 5 and 8 weeks of age appeared to be higher (18%–55%) in 3xTgAD versus non-Tg mice (Fig. 1B), but as these data were derived from group-housed animals no statistical analysis was performed. When housed individually in calorimetry cages at 2 months of age, a significantly greater mean body weight (34%; P < .001, Fig. 1C) and mean 24-hour food intake (17%; P < .05, Fig. 1D) was observed in 3xTgAD mice, when compared with age-matched non-Tg controls. Indirect calorimetry to measure metabolic rate revealed that at 2 months of age, no statistical differences in mean daily VO2 (P > .05, Figs. 1E and H), VCO2 (P > .05, Figs. 1F and I) or RQ (P > .05, Figs. 1G and J) were detected between 3xTgAD and non-Tg mice. During the metabolic monitoring period, both groups of mice exhibited a similar degree of positive energy balance (non-Tg, 5.2 ± 0.3 kcal/d vs. 3xTgAD, 5.4 ± 0.7 kcal/d, P > .05).

Pathological analysis of brains from 2-month-old 3xTgAD mice confirmed the presence of intraneuronal Aβ in the hippocampus, cortex, and amygdala. No hyperphosphorylated tau was detected in the hippocampus, cortex, and amygdala of 2-month-old 3xTgAD mice. No Aβ or tau pathology was observed in control non-Tg mice (data not shown).

3.2. Food intake, body weight, and metabolic rate in 12-month-old 3xTgAD mice

Over a 12-month period, male 3xTgAD mice displayed different body weight (Fig. 2A) and food intake (Fig. 2B) profiles compared with non-Tg mice. In support of the data reported earlier, the body weight of 3xTgAD mice aged between 5 weeks and 4 months was 12%–33% higher (P < .05) than that of non-Tg animals. In contrast, between 7.5–8.5 and 9.5–12 months of age, body weight was significantly lower (by 8%–9% and 11%–18%, respectively) in 3xTgAD compared with non-Tg mice (P < .05). However, despite these biphasic differences in body weight in 3xTgAD mice, the estimated average weekly food intake per
Appendices

E.M. Knight et al. / Neurobiology of Aging xx (2010) xxx

Fig. 1. Food intake, body weight, and metabolic rate in 2-month-old 3xTgAD mice. Weekly body weight (A) and food intake (B) for group-housed male 3xTgAD and non-Tg mice, and body weight (C) and food intake (D) in individually housed mice taken as a 24-hour mean over a 4-day period in calorimetric cages. Oxygen consumption (VO₂) (E), carbon dioxide production (VCO₂) (F), and respiratory quotient (RQ) (G) were measured by calorimetry over a 4-day period in individually housed 3xTgAD and non-Tg mice. White bars represent the light, inactive phase of the day, whereas black bars represent the dark, active phase. The mean 24-hour profile over the 4 days for VO₂, VCO₂, and RQ was calculated and is illustrated in E, F, and G, respectively. Data (for all except B) are mean ± standard error of the mean (SEM) for n = 4 per group. *P < .05, **P < .01 versus non-Tg control mice.

mouse was greater throughout the monitoring period (Fig. 2B), and over this 12-month period, 3xTgAD mice ate 37% more food. Although not accurately assessed, the body length of the 3xTgAD mice did not appear to differ from the non-Tg mice over the 12-month monitoring period.

When housed individually in calorimetry cages, 12-month-old 3xTgAD mice exhibited a significantly higher mean 24-hour food intake (30%; P < .05, Fig. 2D) than non-Tg, despite showing a 15% lower body weight (P < .01, Fig. 2C). Indirect calorimetry over 4 days demonstrated that 3xTgAD mice had a significantly higher metabolic rate as assessed by a greater mean daily VO₂ (24%; P < .01, Figs. 2E and H) and VCO₂ (29%; P < .001, Figs. 2F and I), when compared with non-Tg mice. No significant difference in mean daily RQ was observed between 3xTgAD and non-Tg mice (Figs. 2G and J). When the mean daily energy balance was assessed during the metabolic monitoring period, both groups of mice exhibited a positive energy balance and there was no significant difference between groups (non-Tg, 4.3 ± 1.9 kcal/d vs. 3xTgAD, 5.8 ± 0.8 kcal/d, P > .05).

Pathological analysis of brains from 12-month-old 3xTgAD mice confirmed the presence of intraneuronal Aβ in the hippocampus, cortex, and amygdala and the occasional Aβ-positive plaque in the hippocampus. A small amount of hyperphosphorylated tau was detected in the caudal hippocampus, amygdala, and entorhinal cortex of 12-month-old 3xTgAD mice. No Aβ or tau pathology was observed in control non-Tg mice (data not shown).

3.3. Food intake, body weight, and metabolic rate in 18-month-old 3xTgAD mice

At 18 months of age, 3xTgAD mice had a significantly lower body weight (13%; non-Tg, 40.4 ± 0.8 g vs. 3xTgAD,
Fig. 2. Food intake, body weight, and metabolic rate in 12-month-old 3xTgAD mice. Weekly body weight (A) and food intake (B) for group-housed male 3xTgAD and non-Tg mice, and body weight (C) and food intake (D) in individually housed mice taken as a 24-hour mean over a 4-day period in calorimetric cages. Oxygen consumption (VO\textsubscript{2}) (E), carbon dioxide production (VCO\textsubscript{2}) (F), and respiratory quotient (RQ) (G) were measured by calorimetry over a 4-day period in individually housed 3xTgAD and non-Tg mice. White bars represent the light, inactive phase of the day, whereas black bars represent the dark, active phase. The mean 24-hour profile over the 4 days for VO\textsubscript{2}, VCO\textsubscript{2}, and RQ was calculated and is illustrated in H, I, and J, respectively. Data (for all except B) are mean ± SEM for n = 5–6 per group. *P < .05, **P < .01 versus non-Tg mice.

35.0 ± 0.4 g, P < .01), but increased food intake (14%; non-Tg, 5.6 ± 0.1 g vs. 3xTgAD, 6.4 ± 0.3 g, P < .05) compared with non-Tg control mice. Indirect calorimetry over 4 days demonstrated that 3xTgAD mice had a significantly higher metabolic rate as assessed by a greater mean daily VO\textsubscript{2} (23%; non-Tg, 2,699 ± 79 mL/kg/h vs. 3xTgAD, 3,117 ± 105 mL/kg/h, P < .01) and VCO\textsubscript{2} (24%; non-Tg, 2,678 ± 78 mL/kg/h vs. 3xTgAD, 3,224 ± 104 mL/kg/h, P < .01) when compared with non-Tg mice. No significant difference in mean daily RQ was observed between 3xTgAD and non-Tg mice (non-Tg, 0.99 ± 0.01 vs. 3xTgAD, 0.99 ± 0.01, P > .05). When the mean daily energy balance was assessed during the metabolic monitoring period, both groups of mice exhibited a positive energy balance, and there was no significant difference between groups (non-Tg, 5.14 ± 0.3 kcal/d vs. 3xTgAD, 7.33 ± 1.1 kcal/d, P > .05).

Pathological analysis of brains from 18-month-old 3xTgAD mice confirmed the presence of intraneuronal Aβ in the hippocampus, cortex, and amygdala, and extensive Aβ-positive plaques in the hippocampus. Several neurones positive for hyperphosphorylated tau were detected in hippocampus, amygdala, and entorhinal cortex of 18-month-old 3xTgAD mice. No Aβ or tau pathology was observed in control non-Tg mice (data not shown).

4. Discussion

The present study demonstrates, for the first time in a transgenic mouse AD model, a shift to a hypermetabolic state in aged male animals. Although it remains to be determined whether these changes in metabolic rate are also observed in female mice, these results may help us to
understand the weight loss observed in some AD patients, both before and during significant progression of the disease (Gillette et al., 2007). At 2 months of age, 3xTgAD mice weigh significantly more than non-Tg controls, but display no differences in metabolic rate. However, by 12 months of age, 3xTgAD mice weigh less and have a higher metabolic rate compared with controls, effects that are still present in 18-month-old 3xTgAD mice. Data presented here and by others demonstrate the presence of intraneuronal Aβ in the brains of 2-month-old 3xTgAD mice (Rodriguez et al., 2008). Increases in the number of cells expressing intraneuronal Aβ are seen in 12-month-old 3xTgAD mice, but only occasional Aβ plaques, and a small number of hyperphosphorylated tau-positive neurones are observed. By 18 months of age, a more severe AD-like pathology is noted in 3xTgAD mice in the present study, which is characterized by an increase in the number of Aβ plaques and hyperphosphorylated tau in the hippocampus. These data, therefore, suggest that the changes in metabolic rate reported here start before significant disease pathology (plaques and tangles), but persist with increasing severity and progression of AD-like pathology. However, to determine when and how the changes in metabolism occur, a more detailed analysis of metabolic rate between ages 2 and 12 months is required.

To lose body weight, energy gained from food (energy intake) must be less than energy used (energy expenditure), which leads to a negative energy balance and can result from a decrease in food intake and/or an increase in energy expended (e.g., an increase in metabolic rate and/or activity). Food intake in 3xTgAD mice was higher at 12 and 18 months of age, when they weighed less than control mice. Increased feeding behavior associated with a reduction in body weight has recently been reported in other AD mouse models, which develop amyloid deposition only (Pugh et al., 2007; Vloeberghs et al., 2008). This suggests that the changes reported here in body weight and metabolism in 3xTgAD mice that express both amyloid and tau may be related to the abnormal expression of amyloid. Enhanced appetite in the 3xTgAD mice correlates with clinical findings that food intake in AD patients is usually adequate for their body requirements or even increased (Burns et al., 1989; Keene and Hope, 1997a, b; Niskanen et al., 1993; Smith et al., 1999; Spindler et al., 1996). In addition to an increase in food intake, 12- and 18-month-old 3xTgAD mice had a higher metabolic rate compared with control mice. Despite this increase in metabolic rate, 3xTgAD mice were not in negative energy balance, probably because of the increased food consumption. However, by 12 (and 18) months of age, 3xTgAD mice already weigh less than controls, and measures of energy intake and expenditure in the preceding period would therefore be required to determine energy balance as body weight changes. Furthermore, analyses of body energy content at various stages of development would also be needed, to fully allow assessment of energy balance. A reduction in body weight in the presence of positive energy balance could also result from impairments in gut absorption or a reduction in the amount of metabolizable energy obtained from food. Metabolizable energy decreases if energy lost in urine increases (e.g., if protein use is altered or kidney function is impaired, or in diabetes). It is possible therefore that changes in gut absorption or metabolizable energy are responsible for the lower body weight seen here in 3xTgAD mice aged >12 months, as at these ages they do not display negative energy balance.

A raised metabolic rate can result from several factors, including increases in basal metabolic rate, diet-induced thermogenesis, or physical activity. The hypermetabolism observed in aged 3xTgAD mice is unlikely because of increased physical activity as these mice spend less time moving in the open-field than non-Tg controls (Gimenez-Llort et al., 2007), although measurements of activity over a 24-hour period are still required. Furthermore, the APP/PS1 mouse, which has an increased appetite but reduced body mass, also displays a decrease in physical activity (Pugh et al., 2007). Although increases in diet-induced thermogenesis in response to enhanced calorie intake could be responsible for the hypermetabolism observed in aged 3xTgAD mice, young (2-month-old) 3xTgAD mice also exhibit enhanced food intake but show no changes in metabolic rate when compared with control mice, making this unlikely. Therefore, an increase in basal metabolic rate is most likely responsible, although it is yet to be determined when the switch to the hypermetabolic state occurs in 3xTgAD mice, and whether this coincides with, or is responsible for, the observed differences in body weight.

Sustained increases in basal metabolic rate are seen in other disorders where weight loss is a serious complication, such as cancer, burns, sepsis, and chronic obstructive pulmonary disease (Stephens and Fearon, 2007). The reasons for the hypermetabolic state seen in these diseases, and in the present model, are not fully understood but could be due to many factors. Protein turnover is a key determinant of basal metabolic rate, and as changes in protein concentration have been identified in urine from AD patients, increases in protein use could lead to an increase in metabolic rate (Fonteille et al., 2007). Furthermore, increases in basal sympathetic nervous system activity (Pascualy et al., 2000) and changes in thyroid function (Kapaki et al., 2006), both of which raise metabolic rate, have been reported in AD patients and could therefore be responsible, although this remains to be determined. Alternatively, it is possible that the differences we observe in metabolic rate in 3xTgAD mice are due to abnormal maturation processes, but this would be difficult to test experimentally.

The existence of a hypermetabolic state in AD patients who weigh less than control subjects has been reported previously (Wolf-Klein et al., 1995), although several studies have also shown no change, or even a decrease in metabolic rate (Donaldson et al., 1996; Niskanen et al., 1993; Poehlman et al., 1997; Prentice et al., 1989; Wang et al., 1997). However, many of these latter studies used small sample sizes and heteroge-
ous patient populations (e.g., at various stages and severity of disease), controls were based on predicted calculations of metabolic rate instead of using separate age-matched subjects, and no differences in body weight were reported. As weight loss is not reported in all people with AD (Guerin et al., 2005), it will be important to determine metabolic rate in a homogenous group of AD patients who have already lost weight, or are in a dynamic phase of weight loss.

The present study also shows that 3XTgAD mice weigh more than non-Tg controls early in life. Midlife obesity has been identified as a risk-factor for AD (Kivipelto et al., 2005; Whitmer et al., 2005, 2008; Rosengren et al., 2005), and AD-related pathology and/or behavior in several mouse AD models can be exacerbated by high-energy diets that increase body weight (Ho et al., 2004; Julien et al., in press). Given the early weight increase in 3XTgAD mice, they may represent an ideal model to understand the mechanisms underlying these observations. It is not yet known whether the early increase in body weight in 3XTgAD mice is due to an increase in adiposity (deposition of fat) or accelerated growth, and although no differences in body length were noted in the present study, more detailed analysis is required in future studies.

In conclusion, the results of the present study demonstrate a raised metabolic rate in an experimental animal model of AD. Whether these changes in metabolic rate are responsible for the change in body weight observed in the 3XTgAD mouse model of AD is currently unknown. However, determining whether the switch to a hypermetabolic state occurs, and the mechanisms involved, may potentially help us understand the debilitating weight loss observed in AD.

Conflict of interest

There is no actual or potential conflict of interest.

Acknowledgements

This study was funded by the Medical Research Council and the Research Council, UK. We also acknowledge the support of the local Alzheimer’s Research Trust Network. We are also grateful to Sarah Gumusoglu for invaluable technical assistance.

References


Arendash GW, King DL. 2002. Intra- and intertask relationships in a behavioral test battery given to Tg2576 transgenic mice and controls. Physiology & behavior 75:643-52
Bliwise DL. 1993. Sleep in normal aging and dementia. Sleep 16:40-81
References


Dong H, Yuede CM, Yoo HS, Martin MV, Deal C, et al. 2008. Corticosterone and related receptor expression are associated with increased beta-amyloid plaques in isolated Tg2576 mice. Neuroscience 155:154-63


Fischer MH. 2001. Probing spatial working memory with the Corsi Blocks task. Brain Cogn 45:143-54


Gorospe EC, Dave JK. 2007. The risk of dementia with increased body mass index. Age Ageing 36:23-9


References
References


Holt RI, Byrne CD. 2002. Intrauterine growth, the vascular system, and the metabolic syndrome. Semin Vasc Med 2:33-43


References


