School of Public Health

Enterocyte Beta Amyloid-Lipoprotein Homeostasis: Implications for Alzheimer's Disease Risk

Susan Galloway

This thesis is presented for the Degree of
Doctor of Philosophy
of
Curtin University

March 2015

Declaration

To the best of my knowledge and belief this thesis titled “Enterocyte Beta Amyloid-Lipoprotein Homeostasis: Implications for Alzheimer's Disease Risk” contains no material previously published by any other person except where due acknowledgment has been made. This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

Susan Galloway

March 2015

Acknowledgements

I would like to express my uttermost appreciation for my supervisor, Professor John Mamo, who unequivocally supported me during my Honours in 2005 and throughout my Ph.D. He was a source of inspiration during my honours and it was during his tutelage that I became interested in research and postgraduate studies. I am particularly grateful for his assistance and guidance in manuscript preparation and writing, scholarship applications and writing of this thesis. He remains one of the most interesting and insightful people I have met and his breadth of knowledge spans truly beyond his area of expertise. I will be eternally grateful for the experiences and valuable life lessons he has shared.

I would also like to say a special thank you to Dr. Le Jian who has provided support and many meaningful discussions which were sources of great encouragement. In the laboratory, I was particularly honoured to work with Mr. Russell Johnsen, Dr Stewart Chew, Mr. Columbur Cheung, Mr Karl Fairhurst and Dr Marilyn Bennet-Chambers, who devoted much time and efforts into helping me continuously with technical support, constructive feedback and moral support although they were busy with other work – thank you.

It has been a great pleasure to be walking this path along side my colleagues and dear friends, Dr. Menuka, Dr. Ryu and Miss Virginie Lam. Thank you for the many fond memories and good laughs as well as many meaningful discussions and providing great advice throughout. The laboratory would be lonely if not for you.

I would like to express my heartfelt gratitude to my mother, Yu, for her unconditional love and patience. If not for her instilling in me the importance of learning and education, I may not be where I am today. I would like to thank my father and my family overseas for their continued support. I would like to thank my Grandparents, Scott, Ross, Ricky, Julie and Sarah for their encouragement and support of our young family during challenging times.

I must acknowledge my best friend and soul mate, Matthew James, without whose encouragement, love, support and editing assistance, I would not have completed this thesis. You are the wind beneath my wings. Last but not least, my two beautiful children, Georgia Mae and Wynton Matthew who came along during my Ph.D. Although timing was not the best in the context of my thesis preparation having the two of you was the best thing I have ever done and you are and will remain my greatest source of motivation. This thesis is for my beautiful family.

Abstract

Alzheimer's disease (AD) is a chronic disease marked by progressive loss of mental function resulting in perturbation of normal behaviour and an inability to perform daily tasks. It is estimated that the number of AD cases globally will triple by 2050. The origin of beta-amyloid (Aβ), the key protein found in cerebral plaques associated with AD, has not been established, however the expression of amyloid precursor protein (APP) ubiquitously throughout the body suggests that Aβ could originate from the brain itself, or the periphery, or a combination of different locations.

The aetiology of AD remains unclear due to uncertainty as to where the majority of plaque Aβ originates. Most research has been focussed on the brain as the primary source of Aβ (local production) as the blood-brain barrier (BBB) prevents the entry of most peripheral macromolecules and proteins (including Aβ) into the brain. However, brain samples from deceased AD patients show damage to cerebral blood vessels, increased haemodynamics and the presence of Aβ within the layers of cerebral vessels which is suggestive of possible contribution from peripheral organs.

The increased incidence of AD is predominately related to the ageing population which may or may not have cardiovascular disease, diabetes, insulin resistance and obesity. The second most influential risk factor (after aging) for AD is the presence of an apolipoprotein (apo) E 4 allele. Approximately half of all AD sufferers have one or two apo E4 alleles and more than a quarter of all apo E4 carriers have AD. Apolipoprotein E4 is also implicated in vascular disorders and the presence of such disorders as well as cardiovascular disease increases the likelihood of developing vascular dementia (vAD) and AD.

Compared to the pre-agricultural period (or Paleolithic period), there has been substantial changes in the intake of high calorie foods, sugar, carbohydrate, protein, fat, cholesterol, fibre, dairy, antioxidants and micronutrients (Eaton et al. 2010). The changes in diet being most widely observed in developed or westernised countries where there is a higher risk of secondary metabolic disorders or

degenerative diseases such as obesity, cardiovascular disease and diabetes (Eaton et al. 2010). The prevalence of AD is also positively influenced by greater urbanisation and a growing shift toward western diet and lifestyle patterns. Furthermore diets high in total calories leading to obesity, IR and diabetes are also implicated in AD development and increased incidence of AD may be associated with programming of central hypothalamic energy metabolism.

Epidemiological studies are consistent with the notion that AD is a disease of lipid metabolism and is significantly influenced by dietary fat type as well as the quantity consumed. Grant et al. (2014) found that the higher prevalence of AD in western countries compared to Asia and Africa correlated with increased caloric intake and increased intake of saturated and trans fatty acids. Some middle chain triglycerides however have been shown to have beneficial health effects in terms of CVD and diabetes which are risk factors for AD (St-Onge et al. 2008). Albanese et al. (2009) showed in middle and low-income countries (South America, China and India) that increased fish consumption was associated with a decreased risk of dementia and that dementia was associated with higher intake of meat. Fatty meat as opposed to lean meat is a term used to define cuts of meat which have greater fatty acid content. The distribution of fat in meat commodities includes (intradermal, interdermal or subdermal fats, and in muscle connective tissue and adipose tissue). Fat stored in adipose cells contains mostly triglycerides which contain fatty acids in particular, higher levels of saturated fatty acids (SFAs). The Honolulu-Asia Aging Study showed that Japanese men who migrated from Japan to Hawaii had a much greater risk of developing AD and similar research indicates a greater risk of AD amongst Japanese people living in America compared to those living in Japan. Increased intake of fatty meat and dairy products such as cream and butter that is high in SFAs can increase the risk of AD whilst a Mediterranean diet rich in olive oil that is monounsaturated fatty acid (MUFAs)-rich and fatty fish that is rich in omega 3 (n-3) polyunsaturated fatty acids (PUFAs) can reduce AD risk. Consistent with epidemiological findings, animal models show increased SFA and CH consumption accelerates Aβ production whilst addition of oleic acid (MUFA) and fish oil (n-3 PUFA) to diets reduces the rate of Aβ deposition into cerebral plaques. Cell studies also support these findings and provide additional insight into the intracellular mechanisms involving fatty acids and production of Aβ from APP.

Triglycerides (TGs) are the major type of lipid absorbed by the small intestinal epithelial cells called enterocytes. Within enterocytes, assembly of nascent chylomicrons (CMs) occurs from the addition of TGs, CH, CH esters and phospholipids to apo B48 within endoplasmic reticulum (ER) and Golgi apparatus. Nascent CMs are then transported via the Golgi apparatus to the basolateral membrane and secreted into mesenteric lymph where they enter the circulation via the subclavian vein. Hydrolysis by lipoprotein lipase (LPL) results in the formation of smaller TG-poor and CH and apo E rich CM remnants. These apo E-rich CM remnants participate in rapid clearance by the liver which is mediated by receptor-specific processes (Hussain et al. 1996).

There is evidence that AD and mild cognitive impairment (MCI) patients have greater concentration of Aβ in triglyceride-rich lipoproteins (TRLs) fraction including CM (Mamo et al. 2008). Several studies which measure plasma lipids in AD patients show a significant increase in apo B levels in fasting and non-fasting states and clinical studies show higher levels of apo B in AD patients compared to controls. One study showed in probable AD patients (determined by ICD 10 criteria) who had intake of breakfast (after 24 hour [h] fast) containing 20 g of dairy fat had significantly greater postprandial level of apo B48, a marker for CM. Levels of apo B48 was shown to increase by 400 % in probable AD subjects compared to age-matched controls (Mamo et al. 2008). There is some evidence to suggest that metabolism of Aβ occurs parallel to the metabolism of intestinally derived CM (Mamo et al. 2008). Firstly, there is an increased secretion of soluble amyloid precursor protein (APP) following the ingestion of a fatty meal in healthy individuals; this is consistent with the possibility that dietary fats can influence APP processing, Aβ production and the postprandial Aβ response (Boyt et al. 1999). Secondly, secretion of CM has been shown to be principally enhanced by a fatty meal; there is a transient increase in plasma CM-TG levels which peaks 2-3 h after ingestion (Dubois et al. 1994). The relationship between Aβ metabolism and dietary fat and lipoprotein metabolism provides intriguing evidence that cerebral Aβ plaque could be wholly or in part derived from the small intestines.

A fatty meal contains higher than normal recommended (NH&MRC 2006) dietary intake of less than 35 % total fat (kilojoules) and less than 10 % saturated fat (kilojoules). For example, an average adult woman's recommended intake of 2000

kilojoules (to maintain weight), according to the guidelines, should consume less than 18.6 g total fat and less than 5.4 g saturated fat. For an average man's recommended intake of 2500 kilojoules (to maintain weight), the intake of total fat should be kept within 23.3 g and for saturated fat less than 6.6 g.

The Australian Bureau of Statistics (ABS) reported that 300,000 people were affected with AD in 2011, and global statistics showed that in 2010, 35 million people were affected. Based on current trends, the number of people living with AD will triple by 2050 (ABS 2008, ABS 2012). Urgency is required for the better understanding of AD disease mechanisms in order to prevent the development of AD and delay the disease progression in AD affected persons. Currently the origin of cerebral plaque Aβ is unclear however several key articles suggest plasma Aβ influx in sporadic AD could contribute to cerebral Aβ load. Therefore, the broad aim of this thesis is to elucidate if Aβ can be expressed in the small intestine and if Aβ associates with intestinal lipoproteins. If this is found, the objective to follow would be to determine if intestinal Aβ can be regulated by dietary fat types or influenced by absence of the apo E gene. We hypothesize that Aβ will be present in the enterocytes; at the site of protein synthesis and be associated with intestinal apo B. Consistent with the association of Aβ with lipids, we further speculate that apo E, which can regulate lipoprotein metabolism, may influence intestinal Aβ abundance. Lastly, compared to unsaturated fatty acids, saturated fat diet can contribute to development of AD via greater abundance of intestinal Aβ.

Chapter 1 (literature review) provides a review of the current understanding surrounding pathological mechanisms, epidemiology and risk factors for AD and proposes possible avenues for future research. We hypothesize that the small intestine contributes significant Aβ to the plasma load; a process regulated by dietary fat intake. The small intestine may be the initial organ involved in Aβ production and be of interest in therapeutic interventions in relation to AD. Chapters 2, 3, 4 and 5 are aimed at exploring this idea further.

Chapter 2 (article 1) includes a study aimed to determine the presence of Aβ in the small intestine using wild-type mice fed either low or high SFA diets. Using immunohistochemistry (IHC) approach, this study was the first to locate in situ Aβ within the absorptive enterocytes of the small intestine. We reported stimulatory

effects of dietary SFAs and suppressive effects of fasting on enterocytic Aβ-lipoprotein homeostasis. Furthermore, Aβ expression was shown to occur in the perinuclear location of enterocytes; the location of protein synthesis and lipidation of apo B. Therefore, based on the results that are presented in chapter 2, it is conceivable that the homeostasis of enterocytic Aβ-lipoprotein is influenced by the availability of lipids from dietary sources.

Individuals heterozygous or homozygous for apo E4 are more susceptible to developing AD by 17 % and 43 % respectively compared to apo E2 or apo E3-individuals. The role of apo E isoforms in AD has been extensively reviewed in the literature and the mechanisms of action can include decreased ability to bind Aβ, increased cleavage of APP, increased inflammation and oxidation and increased permeability of the blood-brain barrier (BBB) (Donahue and Johanson 2008, Fryer et al. 2005, Irizarry et al. 2004, Jofre-Monseny et al. 2008, LaDu et al. 1997, Mahley and Huang 1999, Poirier et al. 1993, Refolo and Fillit 2004). Notionally, compared to apo E2 and E3 individuals, apo E4 carriers exhibit altered metabolism of Aβ which increases the propensity for cerebral Aβ toxicity and formation of amyloid plaques. Isoforms of apo E can also be implicated in the transport of lipoproteins derived from the small intestine and liver, which have both been shown to produce Aβ under physiological conditions. Chapter 3 (article 2) discusses the role of apo E in enterocytic Aβ abundance. This study used C57BL6J apo E null (-/-) mice and wild type mice fed either low fat diet containing 4 % (w/w) fat or high fat and CH diet containing 20 % (w/w) fat (predominantly palmitic and stearic acid) and 2 % (w/w) CH for 12 weeks (w). Semi-quantitative IHC was used to quantify and compare the difference in Aβ abundance between groups. The study found high fat + CH feeding significantly increased enterocytic Aβ abundance in both apo E null mice and wild-type mice. In addition, apo E null mice had increased villi height compared to wild-type mice on a low fat diet whilst no effect on villi height was observed under high fat and CH feeding. The findings of this study indicate the presence of apo E can influence the absorption and availability of lipid substrate for maturation of lipoproteins and therefore may modulate enterocytic Aβ via indirect mechanisms.

The current mechanisms of enterocytic Aβ association with postprandial lipoproteins are unclear. Previous observations (chapters 2 and 3) that show Aβ occurring within the perinuclear location of the Golgi apparatus and ER suggest that

Aβ can interact with primordial lipoproteins during the lipidation of apo B during synthesis. Apolipoprotein B is an obligatory component of postprandial lipoproteins derived from the enterocytes. Research presented in chapter 4 (article 3) uses 3-D double immunofluorescence (IF) to investigate the co-association of Aβ with apo B within Golgi apparatus of enterocytes. Colocalization of Aβ with apo B was determined with Manders Overlap coefficient and Pearson's coefficient was used to determine correlation of fluorescent intensities. A significant proportion of Aβ colocalized with intestinal apo B within the perinuclear location and lacteals. Under the influence of high fat diet, colocalization of Aβ with apo B was enhanced consistent with increases in both Aβ and apo B expressions. However, there was no observed relationship between relative abundance of Aβ and apo B with either low fat or high fat diets. The findings presented in this chapter support the notion that Aβ associates with apo B and becomes incorporated into postprandial lipoproteins within enterocytes, and that high fat feeding could exacerbate this process.

It is unknown how the structural differences between fatty acid types affect pathological processes underlying the development of AD. Current evidence in transgenic animal models suggests that differences between fat types can affect rate and severity of amyloidosis in the brain by altering the rate of Aβ production and secretion from cells. For example, palmitic acid and stearic acid (both SFA's) have been shown to increase proteolytic processing of APP leading to an increase Aβ production, whilst oleic acid (MUFA) and DHA/EPA (n-3 PUFA) reduce Aβ production from APP. Palmitic and stearic acids are two saturates that behave quite differently *in vivo* in many different ways, from digestion to various metabolic pathways. In fact most stearic acid is poorly absorbed and that which is absorbed is largely converted to oleic acid. Whether they give the same result in terms of AD is also debatable and requires greater investigation. Current literature does not address the effect of fatty acid type on enterocytic Aβ-lipoprotein abundance, which may be significant in AD and similar disorders. In the article presented in chapter 5, wild-type mice were fed high fat diets (20 % w/w) containing predominantly SFA, MUFA, PUFA or a low fat standard diet and small intestines were collected for immunofluorescence (IF) analysis of Aβ. Results showed diets high in fat increased enterocytic Aβ abundance and effects were not specific to fatty acid type. In summary, the findings presented in chapter 5 indicate SFA, MUFA and PUFA all play a role in secretion and clearance, rather than production of Aβ-lipoproteins.

Alzheimer's disease patients exhibit cerebrovascular disturbances such as sequestration of Aβ within the blood vessels of the brain, proliferation of vascular cells and inflammation. Moreover, plasma-derived proteins are increasingly identified in AD brains. These observations are consistent with the notion of damaged BBB and underlying vascular pathology in AD. The role of dietary fatty acids (SFA, MUFA and PUFA) on BBB integrity was explored in article 4 (appendix B). Interestingly, high SFA feeding caused increased leakage of the BBB demonstrated by increased bi-directional transport of proteins such as S100B and apo B/Immunoglobulin (IgG) which are known to be exclusively present in the brain or blood respectively (Takechi et al. (2010b). Collectively, chronic high SFA feeding results in increased permeability of the BBB and increased sequestration of apo B with Aβ within the arterial walls of cerebral blood vessels (Takechi et al. (2010b). Coupled with previous findings, these results suggest that in the presence of damaged BBB, high SFA feeding increases production and cerebral influx of enterocytic Aβ-apo B-lipoprotein complex. Indeed, co-localization of apo B with cerebral amyloid plaques has been demonstrated in humans and transgenic mouse models of AD (Takechi et al. 2009) which strengthens the possibility of the role of peripheral transport of Aβ-apo B-lipoprotein complex in AD pathology.

Findings presented in the review article (Takechi et al. 2008) as well as in chapters 2 and 3 provide novel insight into the effects of dietary regulation of enterocytic Aβ and the role of lipid substrate availability in enterocytic Aβ-lipoprotein abundance and elucidate an intracellular association between Aβ abundance and postprandial lipoprotein homeostasis."

List of Publications Included

This thesis contains three scientific articles published in peer-reviewed scientific journals. Statements of contribution by co-authors and copyright declarations are provided as Appendix A (page 152).

1. **Galloway S**, Jian L, Johnsen R, Chew S & Mamo JC. (2007) beta-amyloid or its precursor protein is found in epithelial cells of the small intestine and is stimulated by high-fat feeding. *J Nutr Biochem.* 18, 279-284.

   [Impact factor 4.6]

2. **Galloway S**, Pallebage-Gamarallage MM, Takechi R, Jian L, Johnsen RD, Dhaliwal SS & Mamo JC. (2008) Synergistic effects of high fat feeding and apolipoprotein E deletion on enterocytic amyloid-beta abundance. *Lipids Health Dis.* 7, 15.

   [Impact factor 2.0]

3. **Galloway S**, Takechi R, Pallebage-Gamarallage MM, Dhaliwal SS & Mamo JC. (2009) Amyloid-beta colocalizes with apolipoprotein B in absorptive cells of the small intestine. *Lipids Health Dis.* 8, 46.

   [Impact factor 2.0]

List of Additional Publications

Additional publications listed are complementary but not specific to main hypothesis. Statements of contribution by co-authors and copyright declarations are provided as Appendix A (page 152). Articles listed are provided as Appendix B (page 188).

4. Takechi R, **Galloway S**, Pallebage-Gamarallage MM & Mamo JC. (2008) Chylomicron amyloid-beta in the aetiology of Alzheimer's disease. *Atheroscler Suppl.* 9, 19-25.

   [Impact factor 9.7]

5. Pallebage-Gamarallage MM, **Galloway S**, Johnsen R, Jian L, Dhaliwal S & Mamo JC. (2009) The effect of exogenous cholesterol and lipid-modulating agents on enterocytic amyloid-beta abundance. *Br J Nutr.* 101, 340-347.

   [Impact factor 3.3]

6. Pallebage-Gamarallage MM, **Galloway S**, Takechi R, Dhaliwal S & Mamo JC. (2012) Probucol suppresses enterocytic accumulation of amyloid-beta induced by saturated fat and cholesterol feeding. *Lipids.* 47, 27-34.

   [Impact factor 2.6]

7. Takechi R, **Galloway S**, Pallebage-Gamarallage MM, Wellington CL, Johnsen RD, Dhaliwal SS & Mamo JC. (2010a) Differential effects of dietary fatty acids on the cerebral distribution of plasma-derived apo B lipoproteins with amyloid-beta. *Br J Nutr.* 103, 652-662.

   [Impact factor 3.3]

8. Takechi R, **Galloway S**, Pallebage-Gamarallage MM, Lam V & Mamo JC. (2010b) Dietary fats, cerebrovasculature integrity and Alzheimer's disease risk. *Prog Lipid Res.* 49, 159-170.

   [Impact factor 10.2]

List of Additional Publications

Below are a list of additional co-authored publications which are related to this thesis but not included.

9. Takechi R, **Galloway S**, Pallebage-Gamarallage MM, Johnsen RD & Mamo JC. (2008) Three-dimensional immunofluorescent double labelling using polyclonal antibodies derived from the same species: enterocytic colocalization of chylomicrons with Golgi apparatus. *Histochem Cell Biol.* 129, 779-784.

   [Impact factor 2.9]

10. Takechi R, **Galloway S**, Pallebage-Gamarallage M, Wellington C, Johnsen R & Mamo JC. (2009) Three-dimensional colocalization analysis of plasma-derived apolipoprotein B with amyloid plaques in APP/PS1 transgenic mice. *Histochem Cell Biol.* 131, 661-666.

    [Impact factor 2.9]

11. Pallebage-Gamarallage MM, Takechi R, Lam V, **Galloway S**, Dhaliwal S & Mamo JC. (2010) Post-prandial lipid metabolism, lipid-modulating agents and cerebrovascular integrity: implications for dementia risk. *Atheroscler Suppl.* 11, 49-54.

    [Impact factor 9.7]

12. Lam V, Takechi R, Pallebage-Gamarallage MM, **Galloway S** & Mamo JC. (2011) Colocalisation of plasma derived apo B lipoproteins with cerebral proteoglycans in a transgenic-amyloid model of Alzheimer's disease. *Neurosci Lett.* 492, 160-164.

    [Impact factor 2.0]

13. Pallebage-Gamarallage MM, Lam V, Takechi R, Galloway S & Mamo JC. (2012) A diet enriched in docosahexanoic Acid exacerbates brain parenchymal extravasation of apo B lipoproteins induced by chronic ingestion of saturated fats. *Int J Vasc Med.* 2012, 647689.

14. Pallebage-Gamarallage M, Lam V, Takechi R, Galloway S, Clark K & Mamo J. (2012a) Restoration of dietary-fat induced blood-brain barrier dysfunction by anti-inflammatory lipid-modulating agents. *Lipids Health Dis.* 11, 117.

[Impact factor 2.0]

15. Takechi R, Galloway S, Pallebage-Gamarallage MM, Lam V, Dhaliwal SS & Mamo JC. (2013) Probucol prevents blood-brain barrier dysfunction in wild-type mice induced by saturated fat or cholesterol feeding. *Clin Exp Pharmacol Physiol.* 40, 45-52.

[Impact factor 2.2]

Introduction and Structure of Thesis

Background:

The post-mortem presence of cerebral amyloid plaques is diagnostic of AD. Beta-amyloid is the key protein in amyloid plaques and is regarded as the main pathological protein involved in initiation and pathological sequelae of AD. The 39-43 amino-acid length Aβ protein is derived from enzymatic slicing of a large precursor molecule, APP. Production and secretion of Aβ into plasma has been shown to occur as a normal process in cell metabolism (Seubert et al. 1992). Amyloid plaques are produced chronically over time and the deposition of amyloid in plaques is considered a concentration dependent process (Barrow et al. 1992, Wisniewski et al. 1997). Chronic over-production of Aβ from APP results in exacerbated cerebral amyloidosis which can result in an early (< 65 years) and severe form of AD (early onset AD or familial AD). Although increased Aβ production has not yet been linked to the more common form of AD (late onset or sporadic AD), the prevailing theory in AD research suggest a likely involvement of Aβ metabolism (Haass and Selkoe 2007).

The origin of Aβ found in cerebral amyloid plaque is unknown and current knowledge suggests that it could be derived from the brain or periphery or a combination of both. Empirical evidence shows that peripheral Aβ can cross the BBB and contribute to cerebral Aβ load. Although the degree of contribution is uncertain, precipitation and IF of amyloid plaques shows the presence of peripheral proteins in cerebral amyloid plaques. Several studies postulate that plasma proteins have a chaperone role in Aβ transport. In plasma, Aβ has a high tendency to bind to large hydrophobic molecules due to its amphiphilic properties. Kuo et al. (1999) identified that the majority (94 %) of Aβ binds to lipoproteins with high affinity and previous studies show that the binding of free Aβ in the brain is protective against deposition (Wisniewski and Sadowski 2008). Decreases in association of free Aβ with albumin in plasma can contribute to pathology (Yamamoto et al. 2014). In the brain, conversion of monomeric Aβ to an oligomeric form (more prone to aggregation) can be prevented by binding of Aβ to chaperone proteins (Goldgaber et al. 1993). The balance of these chaperone proteins which include lipoproteins is

important and changes in diet or genetics could alter the chaperoning ability of lipoproteins for Aβ. Thus, changes in lipoprotein homeostasis can contribute to pathology of AD by binding to Aβ and thus limiting the deposition of Aβ into amyloid plaques.

In addition, an association between lipid metabolism and AD was discovered when an isoform of apo E, E4 was implicated in a more than doubling of AD risk. Apolipoprotein E is found on low-density lipoprotein (LDL), high-density lipoprotein (HDL) and TG-rich CM and remnant lipoproteins. Apolipoprotein E has several roles in lipid metabolism but its primary role involves transport and clearance of TG and CH from CM remnants by interacting with LDL receptor, LDL-receptor related protein and apo E receptor. Individuals with apo E4 have an altered lipid profile including greater levels of LDL, CH and apo B and less apo E and TG compared to apo E3 and apo E2 containing individuals (Gregg et al. 1986). The increased risk of developing AD associated with apo E4 allele could be linked to the influence of apo E4 on plasma lipid profile.

Moreover, an apo E4 paradox exists: increased intake of SFAs in apo E4 individuals increases AD risk whilst low intake of SFAs in apo E4 individuals decreases AD risk (Petot and Friedland 2004). This study shows that the increased risk of developing AD that is associated with apo E4 can be modified by saturated fat intake. An accumulation of studies has established the significant role of dietary fats and Aβ metabolism in AD risk; however, the precise mechanisms remain elusive.

A group of studies show that dietary fatty acid type can modulate the risk of AD (Barberger-Gateau et al. 2002, Eskelinen et al. 2008, Feart et al. 2009, Freund-Levi et al. 2006, Jick et al. 2000, Kalmijn et al. 1997, Kivipelto et al. 2001, Kivipelto et al. 2002, Luchsinger et al. 2002, Scarmeas et al. 2007, Solfrizzi et al. 1999). Several epidemiological findings (Eskelinen et al. 2008, Kalmijn et al. 1997, Kivipelto et al. 2001, Kivipelto et al. 2002, Luchsinger et al. 2002, Morris et al. 2003, Wolozin et al. 2000) can increase the risk of developing AD and animal models show a diet high in SFAs can increase beta-amyloid production and exacerbate AD pathology (Ho et al. 2004, Refolo et al. 2000, Shie et al. 2002, Sparks et al. 1994). However, in stark contrast, a Mediterranean diet with MUFA as a primary fat source can lower

the risk of developing AD (Feart et al. 2009, Scarmeas et al. 2007) which is thought to be linked to decreased production of Aβ (Amtul et al. 2010). Studies involving consumption of diets high in fish intake (Barberger-Gateau et al. 2002, Freund-Levi et al. 2006, Kalmijn et al. 1996) or low n-6/n-3 PUFA ratio have found such diets (Conquer et al. 2000, Gonzalez et al. 2010) to be associated with enhanced cognitive function and a lowered risk of AD development. The benefits of n-3 PUFA and low n-6/n-3 ration of PUFA in diet was also attributed to decreased production of Aβ (Arsenault et al. 2011, Julien et al. 2010, Lim et al. 2005, Oksman et al. 2006). This thesis addresses the possibility that dietary fats implicated in AD pathology could be a consequence of differential roles of fatty acids in regulating intestinal Aβ.

The hypothesis of this thesis is derived from the host of studies that suggest intestinally derived CMs could be critically involved in the metabolism of Aβ.

Hypothesis:

The small intestine, a lipogenic organ can produce and secrete Aβ, the key protein implicated in AD. Secondly, that intestinal Aβ protein can be regulated by dietary fat, and if so, metabolism of intestinal Aβ is likely to be associated with intestinally derived lipoproteins.

The intended hypothesis addresses the initial part of a broader concept that proposes the possible implications of intestinally and/or hepatically secreted Aβ-lipoproteins in AD pathology; specifically, addressing disruption of the BBB and initiating an inflammatory cascade. Although the content of thesis does not provide empirical evidence that intestinal Aβ is a source of cerebral Aβ, causal links between intestinally derived Aβ and cerebral plaque load will be provided in the general discussion section (chapter 6) and in supporting articles (Appendix B).

Objectives:

Objective 1: To critically review and summarize literature and empirical evidence relating to links between dietary fats, lipid metabolism and AD. (Chapter 1)

Objective 2: To determine whether or not Aβ is expressed in small intestinal enterocytes and if this expression can be modified by a high fat and CH diet. (Chapter 2)
Hypothesis: Aβ is found in the small intestinal epithelial cells (enterocytes) that produce postprandial lipoproteins.

Objective 3: To determine if apo E deletion in the presence of high fat feeding would have an effect on intestinal Aβ in wild-type and apo E gene knockout mice. (Chapter 3)
Hypothesis: Apolipoprotein E can modulate enterocytic Aβ expression and this can be influenced by changes in dietary fat content.

Objective 4: To ascertain whether intestinal Aβ associates with apo B within the enterocytes of the small intestines. Secondly, to further determine if high fat feeding can modulate the degree of association between intestinal Aβ and apo B. (Chapter 4)
Hypothesis: High fat feeding will increase the association between Aβ and apolipoprotein B in enterocytes.

Objective 5: To investigate if fatty acid types (saturated fat, monounsaturated fat or polyunsaturated fat) can differentially regulate intestinal Aβ expression and plasma abundance of apo B and Aβ. (Chapter 5)
Hypothesis: Dietary saturated fat will increase enterocytic Aβ, whilst unsaturated fatty acids will decrease enterocytic Aβ.

Chapter structure:

Chapter 1 – Literature review

This chapter provides a comprehensive review of past and current literature regarding the aetiology of AD in relation to the metabolism of lipid and dietary fatty acids. The review contains a collection of key publications which are considered to be “high-impact” in the area and provide a basis for my hypothesis and objectives.

Objectives addressed:

Objective 1: To critically review and summarize literature and empirical evidence relating to links between dietary fats, lipid metabolism and AD.

Chapter 2 – Determining enterocytic Aβ expression

The content of this chapter is covered by article 1:

Galloway S, Jian L, Johnsen RD, Chew S, Mamo JCL. (2007) B-amyloid or its precursor protein is found in epithelial cells of the small intestine and is stimulated by high fat feeding. *J Nutr Biochem*. 18, 279-284.

Objectives addressed:

Objective 2: To determine whether or not Aβ is expressed in small intestinal enterocytes and if this expression can be modified by a high fat and CH diet.

Chapter 3 – Effect of apo E knockout on enterocytic beta-amyloid expression

The content of this chapter is covered by article 2:

Galloway S, Pallebage-Gamarallage MM, Takechi R, Jian L, Johnsen RD, Dhaliwal SS, Mamo JC. (2008) Synergistic effects of high fat feeding and apolipoprotein E deletion on enterocytic amyloid beta peptide abundance. *Lipids Health Dis*. 7, 15.

Objectives addressed:

Objective 3: To determine if apo E deletion in the presence of high fat feeding would have an effect on intestinal Aβ in wild-type and apo E gene knockout mice.

Chapter 4 – Colocalization of enterocytic beta-amyloid with apolipoprotein B

The content of this chapter will be covered by article 3:

Galloway S, Takechi R, Pallebage-Gamarallage MM, Dhaliwal SS, Mamo JC. (2009) Amyloid-beta colocalizes with apolipoprotein B in absorptive cells of the small intestine. *Lipids Health Dis.* 8, 46.

Objectives addressed:

Objective 4: To ascertain whether intestinal Aβ associates with apo B within the enterocytes of the small intestines. Secondly, to further determine if high fat feeding can modulate the degree of association between intestinal Aβ and apo B.

Chapter 5 – Effect of differential fatty acids on enterocytic beta-amyloid abundance

Objectives addressed:

Objective 5: To investigate if fatty acid types (saturated fat, monounsaturated fat or polyunsaturated fat) can differentially regulate intestinal Aβ expression and plasma abundance of apo B and Aβ.

Chapter 6 – General discussion

The discussion chapter includes several co-authored articles, which discusses the implication of findings, as well as limitation and future direction. In addition, these articles are directly complementing towards my thesis findings.

Abbreviations

3-D	3-dimensional
AA	Arachidonic acid
ABC	Australian Bureau of Statistics
Aβ	Beta-amyloid
AD	Alzheimer's disease
APP	Amyloid precursor protein
Apo	Apolipoprotein
BBB	Blood-brain barrier
BSA	Bovine serum albumin
CTF	C-terminal fragment
CNS	Central nervous system
CSF	Cerebrospinal fluid
CH	Cholesterol
CM	Chylomicron
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
ER	Endoplasmic reticulum
GFAP	Glial fibrillary acid protein
g	grams
h	Hour
HSPG	Heparan sulphate proteoglycan

HF	High fat
HDL	High-density lipoprotein
IF	Immunofluorescence
IgG	Immunoglobulin-G
IHC	Immunohistochemistry
LPL	Lipoprotein lipase
LDL	Low-density lipoprotein
LRP	Low-density lipoprotein receptor-related protein
LF	Low fat
MCI	Mild cognitive impairment
MUFA	Monounsaturated fatty acid
n	Omega
OD	Optical density
PBS	Phosphate buffered saline
PUFA	Polyunsaturated fatty acid
RAGE	Receptor for advanced glycation end product
SFA	Saturated fatty acid
TRL	Triglyceride-rich lipoprotein
vAD	Vascular dementia
VLDL	Very-low density lipoprotein
vWF	Von Willebrand Factor
w	Week

Table of Contents

CHAPTER 1

Chapter 1: LITERATURE REVIEW

This chapter provides a detailed review of past and current literature regarding the aetiology of AD in relation to dietary fats and outlines the potential underlining mechanisms and the possible role that the small intestine plays in the regulation of intestinal lipoprotein absorption and secretion. The literature review references a collection of key publications which are considered “high-impact” in the area of AD research and a significant number of these have provided a foundation for the development our hypothesis and objectives

My own assimilation of current knowledge contained in the literature leads me to believe that AD can be prevented, even amongst those in the population most at risk and that diet is the primary modifiable risk factor in relation to AD risk. Although ageing and the presence of the apo E 4 isoform are regarded as the two most significant risk factors in AD development, an accumulation of epidemiological, animal and cell studies show that ingestion of dietary fats strongly influences AD risk and that SFAs in particular can facilitate increased production and transport of Aβ leading to increased cerebral load. The mechanisms underlying dietary fat influence on AD risk have been thoroughly explored but remain unclear.

1.1 Overview of Alzheimer’s disease

1.1.1 Global prevalence of Alzheimer’s disease

Dementia is a general term describing impairment in cognitive function and can be a consequence of a range of neurodegenerative disorders. Dementia is a disabling illness affecting a person’s ability to function normally on a day-to-day basis. On a global scale, dementia is the primary cause for disability in the elderly, contributing to 13.2 % of the total burden of disease compared with ischaemic heart disease at 14.1 %, lung disease at 12.5 %, glaucoma at 9.4 %, cerebrovascular disease at 7.1 % and rheumatoid arthritis at 4.7 % (World Health Organization 2008). More than half of all dementia cases are clinically diagnosed as AD. The World AD report (Wimo and Prince 2010) estimated that more than 35 million people

are currently living with AD globally and the number of people affected by AD is expected to triple by 2050. The most influential factor in the risk of developing sporadic or late onset (> 65 years) AD is aging and the prevalence of dementia is largely influenced by age.

1.1.2 Dementia and Alzheimer's disease in Australia

In Australia, in 2011 there were 298,000 people living with dementia. Approximately 1 % of the Australian population, 9 % of persons over 65 years of age and 30 % of persons over 85 years of age had dementia. The majority were women (62 %), aged more than 75 years and lived in the community (70 %) as opposed to aged care facilities. Based on current trends, the Australian Bureau of Statistics (ABS) estimates, the number of people living with dementia in Australia in 2050 will triple to 900,000 (ABS 2008, ABS 2012). In 2010, dementia was the third leading cause of death in Australia after ischaemic heart disease and cerebrovascular disease, accounting for 6 % of all deaths and the number of deaths caused by AD was shown to have increased from 3,740 to 9,003 from 2001 to 2010 respectively. This represents a 200 % increase in the reported incidence of AD within a decade (Australian Institute of Health and Welfare 2012).

In terms of costs to the Australian health care system, approximately 552,000 general practitioner visits were related to dementia care in 2010-2011. In the previous year, dementia was diagnosed at a rate of 1 per 100 (or 83,226) hospitalisations with 392,796 dementia specific government funded prescriptions being made. In the same year, $4.9 billion was spent on dementia related health and aged care systems which included about $2 billion directly on dementia; $1.1 billion in residential care facilities and $408 million for aged care services in the community (Australian Institute of Health and Welfare 2012).

1.1.3 Alzheimer's disease symptoms

Alzheimer's disease is a chronic and sinister disease characterised by gradual and progressive loss of cognitive function resulting in behavioral disturbances to episodic memory, language, executive function and visuospatial

abilities. The disease, in most instances, results in accelerated death of the affected person.

Alzheimer's disease was originally described in 1906 by German physician Alois Alzheimer who was treating a 57-year old woman displaying a number of unusual cognitive and psychological abnormalities. Below are three short excerpts (translation with commentary) from Alzheimer's report of the first patient diagnosed with AD -

"[Auguste] was described as being jealous towards her husband and having *'reduced comprehension and memory, as well as aphasia, disorientation, unpredictable behaviour, paranoia, auditory hallucinations, and pronounced psychosocial impairment*' (Maurer et al. 1997) and *"general loss in intelligence"* (Strassnig and Ganguli 2005);

"Although this was true, Auguste was physically functioning: '*The gait is undisturbed; she uses her hands both equally well. The patellar reflexes are present. The pupils react. Slightly rigid radial arteries, no enlargement of cardiac dullness, no protein*" (Strassnig and Ganguli 2005); and

Deterioration of her condition as described by Alzheimer - "*The patient was eventually completely dull; lying in bed with legs pulled up; had let go under her and developed decubitus despite all care."* (Strassnig and Ganguli 2005). Auguste D was admitted to hospital on November 25 1901 and died on April 8 in 1905.

Episodic memory associated with the hippocampus and entorhinal cortex areas is normally the first cognitive skill to become affected in AD. The loss of episodic memory in AD could be due to improper consolidation or storage of new information, thus making learning of new information difficult (Chen et al. 2001, Lange et al. 2002, Small et al. 2000). The inability to "think", for example: problem solving, working memory (focus and attention), concept formation and cue-directed behaviour also occur early in the disease process (Chen et al. 2001, Perry and Hodges 1999).

It is important to note that decline in mental function is a normal physiological phenomenon in aging and is common amongst the elderly, but more rapid and severe rates of decline are evident in MCI and AD. There is currently no definitive diagnosis for AD apart from the post-mortem examination of the brain and confirmation of the presence of amyloid plaque, neurofibrillary tangles (NFTs) and other cerebral changes associated with cognitive impairment.

Mini-mental state examination is a standard questionnaire for determining dementia however it does not differentiate dementia types. Mild cognitive impairment can predict the later development of AD however currently the early diagnostic markers for AD are lacking. Alzheimer's disease is thought to be irreversible after a certain point in the disease thus early diagnosis of AD is critical in developing appropriate medical interventions and slowing the disease progression. Currently, markers and cognitive assessments to aid in the characterisation of a pre-clinical phase of AD are being investigated with the aim of developing a screening tool for cognitively normal patients for the later development of AD (Lazarczyk et al. 2012).

The current enigma surrounding AD pathogenesis contributes to a lack of effective strategies for management and cure of AD. There is currently no cure for AD except for compensatory treatment with cholinesterase inhibitors and N-methyl-D-aspartate receptor antagonists for synapse loss prevalent in AD and for symptomatic treatment with antipsychotics, antidepressants and anticonvulsants. Clinical trials of these treatments have shown mild to no improvement in cognition with little evidence of further efficacy past 12 months treatment duration. Proposed agents which can alter the course of the disease progression have been trialed but are currently inconclusive and some awaiting third phase results (Ballard et al. 2011).

1.1.4 Overview of Alzheimer's disease pathology

Hallmark AD pathology is characterised by post-mortem findings of amyloid or "senile" plaques, NFTs (figure 1), and accompanying microvascular changes (Mattson 2004). These pathological characteristics or 'changes' were first described in 1906 by Alois Alzheimer who upon conducting a post mortem histological brain

examination observed brain atrophy, peculiar "stainable" lesions that were *"refractory to dyes"* (congophilic amyloid plaques) and Bielschofsky silver-positive neurofibrils.

Alzheimer also noted atherosclerotic changes in the patient's blood vessels and Strassnig and Ganguli (2005) described the vessel changes as *"signs of excess proliferation in the endothelium here and there; also a neovascularization."*, However, others report no significant signs of atherosclerosis in AD but *"*some regressive alterations of the arterial wall*"* Perusini report translation by Maurer et al. (1997).

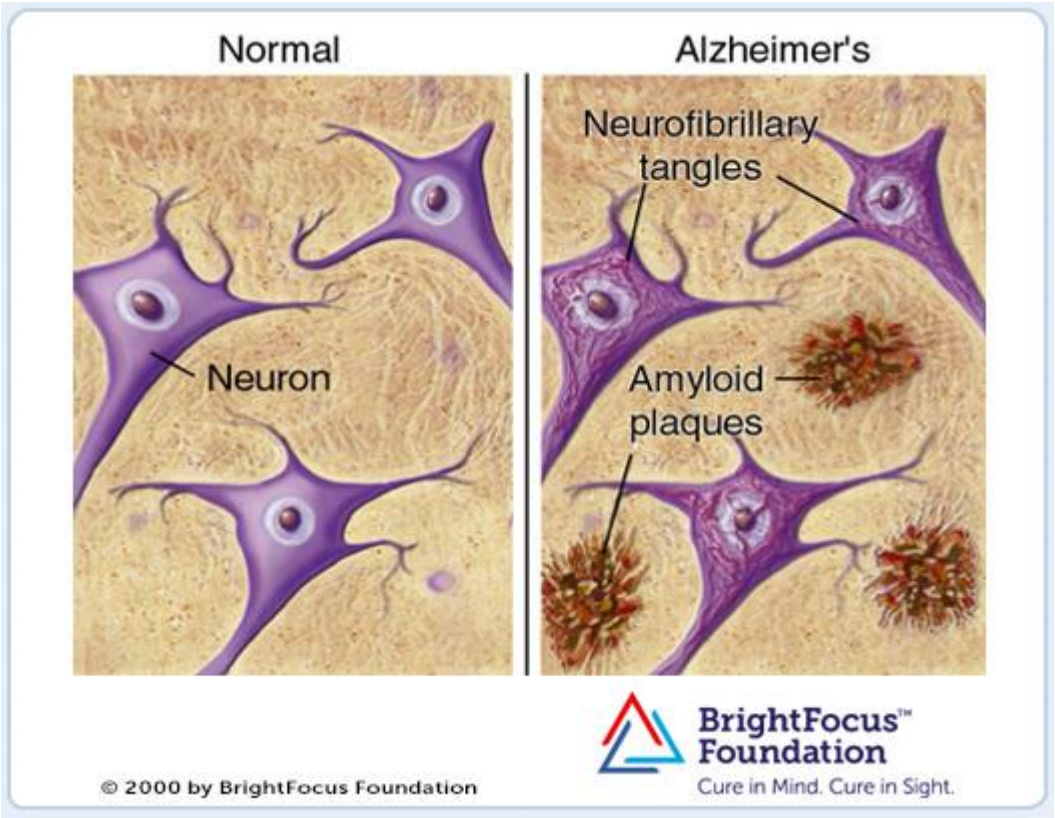


Figure 1. Depiction of brain from individual without Alzheimer's and individual with Alzheimer's showing the abnormal presence of neurofibrillary tangles and amyloid plaques.

Source: The medical illustration is provided courtesy of Alzheimer's Disease Research, a BrightFocus Foundation program.
http://www.brightfocus.org/alzheimers © 2014

1.1.4.1 Amyloid (senile) plaques

Amyloid plaques are amorphous, insoluble and congophilic and contain an abundance of Aβ and other proteins. Amyloid plaques are 50-100 μm in diameter and are found surrounded by activated microglia, reactive astrocytes, and dystrophic axons and dendrites (Barnham et al. 2003). Two forms of amyloid plaques are characterised: diffuse plaques or semi-soluble oligomers which are the result of deposition of soluble Aβ onto extracellular brain matrices (Harper and Lansbury 1997, Teplow 1998); and mature insoluble plaques which are thought to evolve from diffuse plaques (Blessed et al. 1968, Perry et al. 1978) and are considered the hallmark late-stage pathological indication of AD. The conditions under which conversion of diffuse plaques to mature plaques occurs is currently not well understood; however, an increase in soluble Aβ has been shown to result in concentration dependent deposition into the brain (Esler et al. 1996).

Attachment and addition of soluble Aβ to the brain matrices forms insoluble fibrils and is considered to be a concentration dependent process (Nichols et al. 2002). Morphologically, diffuse plaques contain less Aβ and appear as “partially amorphous pre-amyloid fibrils”, whereas the amyloid rich mature plaques have many thick bundles of apparent “amyloid fibrils” (Ikeda et al. 1990).

Brain inflammation as evidenced by the presence of activated microglia, increased cytokines and acute phase and complement proteins has been found to be associated with amyloid plaque development (Akiyama et al. 2000) and the presence of amyloid plaques in the cortex and hippocampus areas responsible for memory and learning indicates that amyloid plaques are associated with neurodegeneration. Although diffuse plaques do not appear to trigger inflammation nor have they been associated with dystrophic neuritis compared to mature plaques which have been associated with a variety of inflammatory components and damaged neurons and neurites. Diffuse plaques are found in brains of cognitively intact individuals of varying ages and therefore are not considered a central neurotoxic component in AD (Blessed et al. 1968).

Despite the pathological significance of Aβ, increased quantities of diffuse and mature plaques have not been shown to correlate with increased levels of cognitive decline (Lue et al. 1999); however, a correlation has been established between elevated levels of soluble Aβ in brain and cognitive decline (Naslund et al. 2000). Levels of soluble Aβ versus insoluble Aβ distinguish AD from normal and pathological aging (Wang et al. 1999). Cognitive decline in AD may therefore function as a consequence of other pathological events other than from plaque number, at least in early disease process.

Presentation of cerebral amyloid plaques may otherwise indicate that soluble Aβ concentration in the brain is higher than an amount that is metabolically sustainable. This idea is therefore controversial to the previous notion that mature plaques were pathogenic. Treusch et al. (2009) proposed another explanation and suggested that mature amyloid plaques may act as a “sink” for soluble Aβ and thus presence may indicate the extent of exposure to the brain rather than measure of neurodegeneration. Although insoluble Aβ plaques can confer protection via the proposed “sink” mechanism, exacerbated deposition of Aβ into diffuse plaques causes conversion of soluble oligomeric Aβ into insoluble amyloid fibrils which have been shown to initiate inflammation and therefore contribute to pathology via a separate pathway. It is reasonable to assume that the balance between cerebral soluble Aβ oligomers and Aβ in plaques is important in maintaining neuronal cells and synaptic function. Chronic over-exposure of the brain to soluble oligomeric Aβ is also likely to be realized as an increase in amyloid plaque burden.

Changes in Aβ concentration in the brain may be caused by alternation of the balance between production, influx, efflux and degradation of Aβ. Therefore, elucidating possible mechanisms underlying Aβ metabolism in the context of oligomeric and plaque formation metabolism is potentially important for considering therapeutic targets and preventative interventions.

1.1.4.2 Neurofibrillary tangles

Neurofibrillary tangles are intraneuronal bundles of paired helical filaments caused by the hyper-phosphorylation of tau protein (Alonso et al. 1996). This abnormal form of tau reduces the ability to bind microtubules leading to cytoskeletal

damage and cell death (Arendt et al. 1998). Neurofibrillary tangles can also be triggered by Aβ via hyperphosphorylation of microtubular associated protein (Busciglio et al. 1995). Neurofibrillary tangles and cerebral inflammation occur in other neurological disorders and appear to be consequential and responsive to Aβ deposition in the brain and the maturation of amyloid plaques. Studies demonstrate that Aβ deposits in the brain early in the disease process and before the appearance of NFTs (Iwatsubo et al. 1994, Lippa et al. 1998, Rapoport et al. 2002, Younkin 1995), and NFT's are thought to be triggered by Aβ (Hardy et al. 1998).

1.2 Beta-amyloid protein in Alzheimer's disease

1.2.1 Overview

Since Alois Alzheimer's original observations, the understanding of the morphological and chemical processes involved in AD neuropathology has evolved considerably. In 1983, Aβ protein was identified as the main component of amyloid brain plaques and research has since determined Aβ protein as the main protein involved in AD pathology (Allsop et al. 1983, Glenner and Wong 1984, Haass and Selkoe 2007, Klein 2002, Masters et al. 1985, Salminen et al. 2009, Tanzi and Bertram 2005, Walsh and Selkoe 2007). Samples of AD affected brains show the widespread presence of Aβ in areas including in the cerebral cortex and the hippocampus as well as the cerebellum, brainstem, basal ganglia, amygdala and to a lesser extent, the diencephalon (Schmidt et al. 1994).

The current dominating hypothesis regarding AD pathology is known as the 'amyloid cascade hypothesis' and supports the key role of Aβ in AD (figure 2). The hypothesis postulates that an imbalance in the normal metabolism of Aβ causes increased cerebral amyloidosis triggering a number of cellular events that lead to cell stress, dysfunction or loss and subsequent cognitive decline (Selkoe 2001). Consistent in current literature is the idea that Aβ deposition occurs early on in the disease process, possibly decades before onset of symptoms. Therefore, understanding the process of Aβ accumulation in the brain is of great importance.

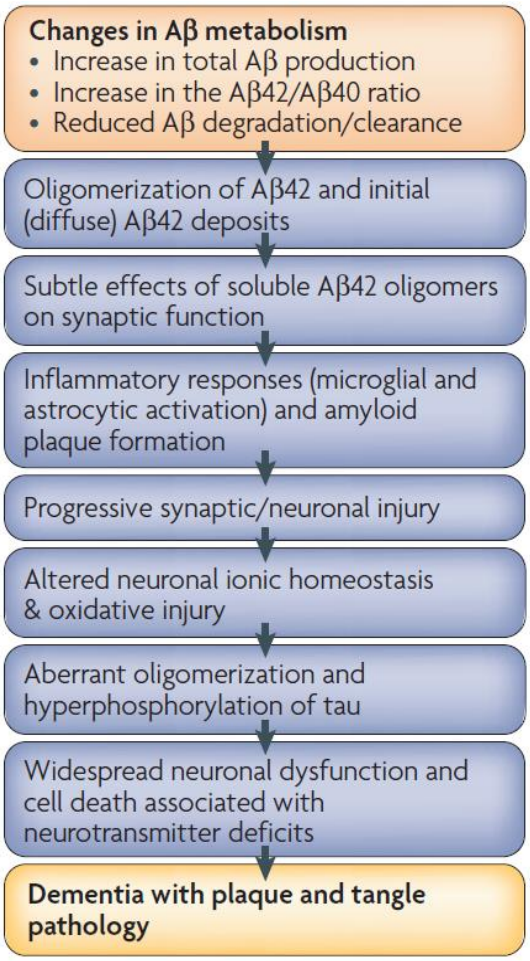


Figure 2. According to The Amyloid Cascade Hypothesis, the sequence of events leading to Alzheimer's disease is initiated by Aβ protein.

Source: Adapted from Box 2, Haass and Selkoe 2007 (License number: 3463090338026).

1.2.2 Toxicity of soluble beta-amyloid

In support of the possibility that soluble Aβ is neurotoxic, animal models show that soluble amyloid oligomers are toxic to synapses and directly cause losses in long-term potentiation in rodent hippocampus (Shankar et al. 2008). Oligomerization of Aβ into dimmers, trimers (Walsh et al. 2002), photofibrils (Harper et al. 1999) or insoluble fibrils (Lorenzo and Yankner 1994, Pike et al. 1993) can mediate the toxicity of Aβ. Oligomeric Aβ and not monomeric forms of Aβ have been shown to be toxic to cultured neurons and synaptic function. A number of studies

show toxic effects of Aβ on long-term potentiation by causing endocytosis of NMDA receptors of postsynaptic membranes (Snyder et al. 2005).

In addition, Aβ can also disrupt calcium homeostasis by causing an increase in influx and release from intracellular compartments (Demuro et al. 2005) causing intracellular damage of endoplasmic reticulum, synapse and calcium-related proteins. Extracellular Aβ deposits have shown to activate microglia via binding to scavenger receptors such as advanced glycation end products (RAGE). Formation of oligomeric Aβ, in particular the longer and more hydrophobic Aβ42, has been shown to nucleate and bind to extracellular matrices of the brain including heparin sulphate proteoglycans. In addition, mature amyloid plaques can activate complement pathway via binding to complement proteins (Eikelenboom et al. 2006) supporting the role of Aβ in initiating an inflammatory response.

1.2.3 Origin of beta-amyloid

Beta-amyloid peptide of 37-43 amino acids lengths are derived from proteolytic processing of 100-140 kDa APP (Kang et al. 1987). Amyloid precursor protein is a type-1 transmembrane protein that is embedded within the cell membrane with N-terminal tails protruding into the lumen and C-terminal within the cytosol (Kang et al. 1987). Amyloid precursor protein cleavage by α- or β-secretases and subsequently, γ-secretase (Sherrington et al. 1995, Sinha et al. 1999) produces soluble APPα and soluble APPβ respectively. Cleavage of APPβ by γ-secretase complex (containing presenilins) yields soluble oligomeric Aβ whereas cleavage of APPα produces a by product known as p3 and the latter pathway prevents Aβ production (figure 3). These are respectively designated amyloidogenic and non-amyloidogenic pathways. Soluble Aβ forms are present at very low, but detectable levels in a normal brain (Tabaton et al. 1994).

Following the amyloidogenic proteolysis of APP, Aβ40 and Aβ42 are secreted into extracellular space. Both Aβ40 and Aβ42 are pathological forms in AD during different stages of the disease progression. Immunopositive Aβ42 has been found in early diffuse plaques (while no Aβ40 was present) (Iwatsubo et al. 1994) whilst Aβ40 seems to be abundant in mature plaques. Soluble Aβ can be detected in plasma, CSF and neuronal supernatant (Busciglio et al. 1993, Haass et al. 1992,

Koudinov et al. 1994, Koudinov et al. 1996b, Seubert et al. 1992, Shoji et al. 1992). Plaque and capillary deposits of Aβ are homologous to soluble Aβ (Busciglio et al. 1993, Vigo-Pelfery et al. 1993) which indicates the soluble Aβ in plasma and cerebrospinal fluid (CSF) can both contribute to plaque Aβ.

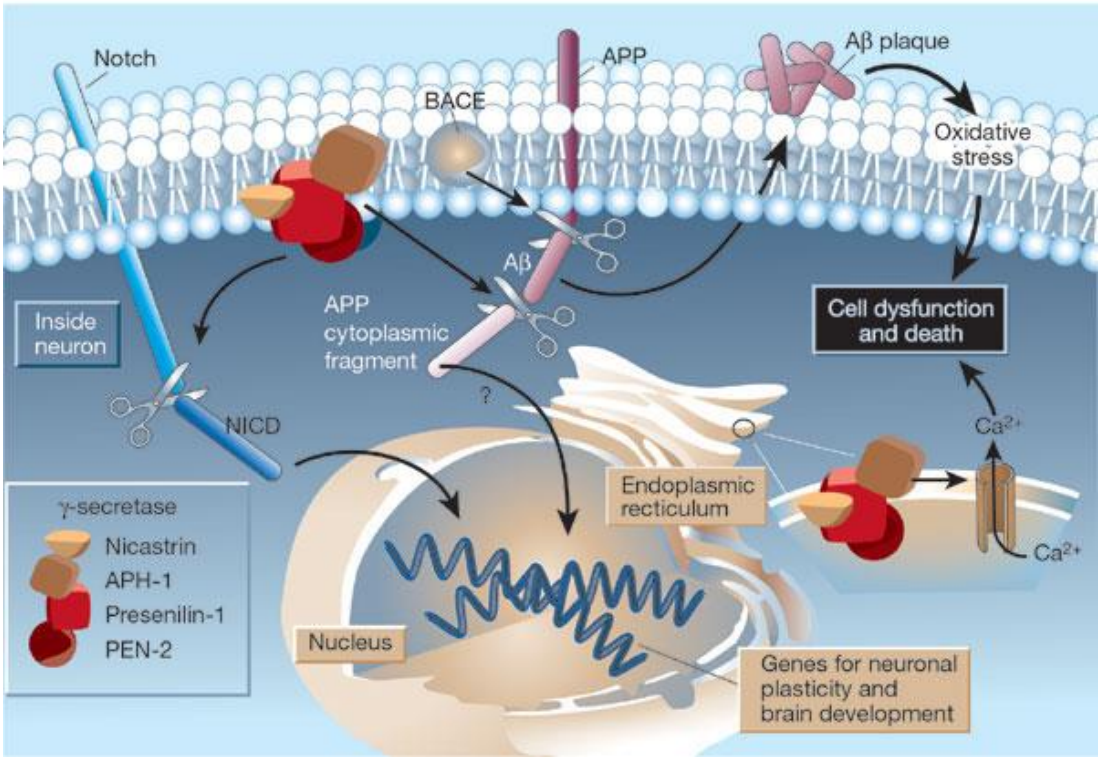


Figure 3. Production of beta-amyloid. Image shows the sequential cleavage of APP in membrane and intracellular compartments to produce Aβ in neuron.

Source: Adaption from Figure 1, Mattson 2004 (License number: 3463110683913)

The ubiquitous genetic expression of APP in various tissues including the brain, heart, kidneys, liver, intestines and adrenal glands and presence of Aβ in CSF, plasma and body fluids indicate plaque Aβ could originate from the brain or periphery or a combination of both (Davis-Salinas et al. 1995, Golde et al. 1990, Yasojima et al. 2001). The relative impermeability of the BBB (Clifford et al. 2007) and high expression of APP in brain (Yasojima et al. 2001) supports a primary cerebral source of plaque Aβ. Other evidence shows however that deposits of Aβ in plaques are located close to or directly on blood vessels which indicate a possible peripheral contribution to cerebral plaque Aβ.

1.2.4 Over production of beta-amyloid in Alzheimer's disease

Neuronal cell production of Aβ is considered a normal and constant event and does not contribute to pathology when the balance between production and clearance of Aβ is well maintained (Selkoe et al. 1996). In cognitively intact individuals, brain soluble Aβ is present within neurons and, in lower concentrations, interstitial space (Andreasen and Blennow 2002, Seubert et al. 1992). Currently, the function of APP and Aβ is not known. There are many major theories includes functional involvement of APP in cell growth and proliferation, function as a cell-surface or extracellular matrix receptor and in regulation of blood clotting (Dawkins and Small 2014). Functions of Aβ may include regulation of lipid metabolism (Kontush et al. 2004), neuronal physiology including cellular transport and apoptosis (Li et al. 2007), and feedback regulation of synaptic transmission (Esteban 2004).

Genetic mutations of APP lead to an abnormal proteolysis of APP and increase in Aβ production resulting in an earlier onset (familial) and more severe form of AD (St George-Hyslop 2000). Conversely, a mutation in APP that causes decreased Aβ production is protective against AD development (Jonsson et al. 2012). Overproduction of Aβ from APP is the key event causing familial AD but less likely in the more common late onset or sporadic form of AD that constitutes 99 % of AD cases (Bateman et al. 2012).

On the contrary, production of brain Aβ in sporadic AD has also been studied and evidence shows little increase in Aβ40 and Aβ42 (Lewczuk et al. 2010) and β-secretase (BACE-1) activity (Zetterberg et al. 2008) in proteolytic processing of APP meaning that other pathological mechanisms are likely to be implicated. Determining the processes of amyloid production from APP and the various pathways which guide the preference towards non-amyloidogenic processing of APP is of great interest in understanding mechanisms of Aβ production in relation to AD.

1.2.5 Inadequate clearance of beta-amyloid from the brain in Alzheimer's disease

Rapid clearance of Aβ from the brain is indicative that high concentrations of soluble Aβ in the brain may not be well tolerated and is consistent with reports of Aβ

induced neurotoxicity. It has been postulated that aberrant clearance of Aβ via transport across the BBB could contribute to accumulation of Aβ (Zlokovic et al. 1993). Clearance of Aβ from the brain can take place from periarterial spaces and drain into venous circulation (Weller et al. 1998). However, a study showed that Aβ clearance from the brain is rapid. Injection of radio labelled Aβ40 into lateral ventricles of Sprague-Dawley rats showed rapid clearance of Aβ peptide, clearing 30 % after 3 min and 70 % after 10 min (Ghersi-Egea et al. 1996). Considering the rate of CSF and Aβ removal from the brain, an aberration in Aβ clearance which causes AD pathology is less likely.

Based on non-significant changes in Aβ40 and Aβ42 production and rapid clearance of Aβ and turnover of CSF, it is less likely that production and clearance is compromised in AD. However, it cannot be excluded either. Studies of CSF Aβ levels have been largely controversial and are complicated by a number of other factors. Firstly, production of Aβ increases during aging, but will decrease as Aβ nucleates in the brain during AD processes. A recent longitudinal study shows changes in CSF Aβ appears 25 years prior the onset of symptoms in AD cases. Levels of Aβ42 and Aβ40 increases with aging, whilst in subjects where cognitive impairment and AD eventuated, Aβ levels slowly decline (Bateman et al. 2012). Interestingly, Aβ42 declined earlier in the disease process and levels remained constant after onset of symptoms whilst Aβ40 declined later in the disease process and levels continued to decline after the onset of symptoms (Bateman et al. 2012). This study was done in familial AD cases; however, histopathological brain pathologies of sporadic and familial cases are virtually indistinguishable indicating similar pathologic processes.

1.2.6 Section summary

Presently, the origin of Aβ found in cerebral plaque has not been established; however, the presence of Aβ in body fluids including blood, CSF, blood vessels and cell supernatant suggests that plaque Aβ could originate from the brain, the blood or both. Beta-amyloid could cause cognitive decline in its soluble form and/or deposit in extracellular matrices of the brain and initiate a pathological cascade of events which have been described in the ‘amyloid cascade hypothesis’. It appears that accumulation of soluble Aβ can lead to toxicity and plaque

development, which over time causes inflammation, oxidation and the appearance of NFTs. Formation of amyloid plaques is a chronic process which depends on a number of conditions including the overproduction of cerebral Aβ and/or inadequate clearance of Aβ from the brain. Production of cerebral Aβ does not increase significantly in sporadic AD but does in familial cases and together with evidence of rapid clearance of Aβ from the brain, suggests that cerebral Aβ could be due to accumulation from another source, notably from plasma. However, few have explored this possibility further as transport across the BBB is considered limited due to structural architecture of cerebral capillary vessels.

1.3 Cerebral vascular and blood-brain barrier changes in Alzheimer's disease

1.3.1 Role of blood-brain barrier in bi-directional transport of beta-amyloid

The blood vessels which line the brain and spinal cord are different from the rest of the body as they are additionally lined by cells of the brain and together are collectively known as the BBB. The BBB is comprised of several cell types including endothelial cells of the capillaries (smooth muscle cells in arterioles and arteries), astrocytes, pericytes as well as basement membranes. Endothelial cells are the first line of defence and are structurally aligned very tightly. These cell to cell junctions are abundant in small units of tight junction proteins, occludin, claudins and junctional adhesion molecules (Abbott et al. 2010, Ballabh et al. 2004). In addition, astrocyte foot processes are involved in modulating endothelial tight-junction proteins and the structural integrity of the endothelium (Balabanov and Dore-Duffy 1998, Zlokovic 2005); however, there is some evidence that pericytes may also influence endothelial cell function.

The BBB is generally impermeable to many plasma proteins, macromolecules, peptides, pro-inflammatory molecules and neurotoxic products (Hawkins and Davis 2005, Mayhan 2001, Risau et al. 1998), allowing only selective influx of molecules via diffusion or active transport mechanisms. Capillary vessels generally prevent blood-to-brain extravasation of peripheral Aβ, although some

transport occurs via RAGE (Kandimalla et al. 2005, Poduslo et al. 1999). Maintenance of barrier function for cerebral capillary vessels is pivotal for maintaining synaptic signal and neuronal plasticity (Nedergaard et al. 2003, Newman 2003, Zlokovic 2005).

Studies of BBB in normal individuals show that under conditions where cerebral blood vessels are intact and functioning normally, plasma-derived Aβ relative to total cerebral load is probably low (Poduslo et al. 2001). However, an increasing number of studies show that BBB damage and altered haemodynamics in AD patients can contribute to an increased influx of plasma components including Aβ. Indeed, the aberration of cerebral blood vessels in AD was first observed and reported by Alois Alzheimer himself - *"large vessels show atherosclerotic changes"* and *"some regressive alterations of the arterial wall"* (initial reports of Alois Alzheimer (1907) and Perusini (1911) was translated from German to English by Maurer et al. (1997).

Whilst current understanding of bi-directional transport of Aβ is limited (Deane et al. 2003, Shibata et al. 2000), entrance of Aβ into the brain can occur via endothelial cells protein RAGE (Deane et al. 2003, Donahue et al. 2006). Yan et al. (1996) found binding of RAGE to Aβ and that the binding site is dissimilar to advanced glycation end products (AGEs). This study also showed that the binding of Aβ to RAGE was inhibited in a concentration dependent matter by addition of AGE albumin or amphoterin. In addition, low-density-lipoprotein receptor-related protein-1 (LRP-1) binds to Aβ within the junctions of endothelial cells of the capillaries of the brain. Transport via LRP-1 primarily mediates efflux rather than influx (Deane et al. 2009, Deane et al. 2005, Donahue et al. 2006). Some consider the balance between RAGE and LRP-1 is indicative of the dominant brain-to-blood efflux of Aβ transport (Deane et al. 2009, Deane et al. 2008, Donahue et al. 2006, Ye et al. 2005).

1.3.2 Role of vasculature disturbance in Alzheimer's disease pathology

The presence of underlying vascular risk factors and cardiovascular disease increases the likelihood of developing vAD and AD (Breteler 2000, Luchsinger and Mayeux 2004). Vascular risk factors include: hypertension, high CH, high TGs, stroke and ischemic attacks (Honig et al. 2003, Vermeer et al. 2003), metabolic

syndrome (Kalaria et al. 2012), diabetes mellitus (Ott et al. 1999, Peila et al. 2002) diabetes, (Strachan 2003) and atherosclerosis. The presence of one or more risk factors can increase the risk of developing AD by between 1.2-4 times with the presence of three or more risk factors showing the greatest risk (Kalaria et al. 2012, Tschanz et al. 2013). Obesity alone has been shown to increase the risk of AD by 2-5 times (Gustafson 2008). The emergence of vascular risk factors typically in mid-life (Kivipelto et al. 2001) demonstrates the possibility for early onset of vascular changes that are present in AD.

The clinical importance of blood vessel integrity and cerebral blood flow (haemodynamics) changes in AD pathology has been increasingly studied. Cerebral amyloid deposits or cerebral amyloid angiopathy is evident in 80 % of AD patients (Thal et al. 2008) and 30 % of AD subjects show evidence of cerebral haemorrhage or micro-emboli (Pettersen et al. 2008). Cerebral blood flow is altered in AD patients with some reporting hypoperfusion (Johnson et al. 2005) with lower blood flow velocity (Ruitenberg et al. 2005). Aberrant blood flow in AD could be caused by the presence of atherosclerotic lesions. Subjects with atherosclerosis are three times more at risk of developing dementia (van Oijen et al. 2007). Atherosclerotic plaques present in leptomeningeal arteries and the main arteries supplying blood to the brain (circle of Willis) are more severe in AD cases compared to nondemented controls (Beach et al. 2007, Kalback et al. 2004, Roher et al. 2003).

1.3.3 Plasma beta-amyloid can damage cerebral vasculature including the blood-brain barrier

Several studies show that circulating Aβ could compromise vascular and specifically BBB integrity (Jancso et al. 1998, Su et al. 1999), damaging vascular endothelium (Thomas et al. 1996) and smooth muscle cells (Crawford et al. 1998) and altering the vasoactivity of cerebral vasculature by increased blood pressure (Arendash et al. 1999), decreased blood flow and increased vascular resistance (Suo et al. 1998). The mechanisms behind Aβ ‘toxicity’ towards vascular integrity have been considered. The vasoactive property of Aβ was first demonstrated by Thomas et al. (1996) when addition of synthetic Aβ to the aorta enhanced vasoconstriction and decreased the relaxation response. Crawford et al. (1997) also showed that vasoconstrictive properties of both Aβ40 and Aβ42 were immediate and

dependent on free radical production. Indeed, decreased cerebral blood flow in frontal, temporal, parietal, and posterior parietal cingulated cortices was observed in AD patients compared to age matched controls (Alsop et al. 2000). A recent article attributed decreased cerebral haemodynamics to impairment of cerebral auto-regulation of blood flow in a mouse model of AD (Claassen and Zhang 2011). The latter observation was confirmed in a human study where cerebral autoregulation was found to be less effective (greater amount of brain pressure fluctuation) compared to age-matched controls (Meel-van den Abeelen et al. 2014). Collectively, these studies indicate that Aβ could initiate cerebral vascular changes and altered brain haemodynamics contributing to the vascular changes observed in AD.

1.3.4 Section Summary

The ubiquitous genetic expression of APP in various tissues (Davis-Salinas et al. 1995, Golde et al. 1990) and high concentration of Aβ in blood (Seubert et al. 1992) coupled with the presence of damaged cerebral blood vessels in AD individuals indicates that peripheral Aβ could contribute to brain Aβ load (Zlokovic et al. 1993). Taking into consideration these studies, it is possible to conclude that cerebral amyloidosis can be exacerbated under circumstance where the BBB is less intact and thus allowing a greater influx of peripherally derived Aβ.

1.4 Lipid metabolism and Alzheimer's disease

1.4.1 Relationship between apolipoprotein E4 and Alzheimer's disease risk

The second most influential risk factor after aging for AD is the presence of an apo E, E4 allele. Apolipoprotein E4 allele has been associated with greater risk of both early onset (or familial) and late onset (sporadic) AD (Corder et al. 1993, Farrer et al. 1997, Saunders et al. 1993, Strittmatter et al. 1993a, Ye et al. 2005) and is present in half of all AD subjects (Farrer et al. 1997).

Apolipoprotein E is a 35 kDa 299 aa long protein produced in the liver and brain, and responsible for cholesterol and TG redistribution and metabolism (Mahley

1988, Mahley and Huang 1999, Mahley and Rall 2000). There are three main isoforms of apo E in humans differing in their primary structure: apo E2 (Cys112 and Cys158), E3 (Cys112 and Arg158) and E4 (Arg112 and Arg 158) (Weisgraber 1994).

Apolipoprotein E is critically involved in transport, redistribution and clearance of CH and TG from lipoproteins (Mahley 1988, Mahley and Huang 1999, Mahley and Rall 2000). Apolipoprotein E is found on all CM and CM-remnants and the level of apo E present on the molecules may vary from the fasted to fed state. More apo E is found on TG-depleted remnants of hepatically derived VLDL and postprandial lipoprotein CMs. Apolipoprotein E is a ligand for apo B100/E receptor and therefore modulates the binding of CM remnant or LDL remnant particles which contain CH and remaining TG after lipolysis. Individuals with apo E4 alleles have different lipid profiles including greater levels of LDL-CH and apo B and less apo E and TG compared to individuals with apo E3 and apo E2 (Gregg et al. 1986). Differences in plasma lipid profiles have been attributed to differences in apo E association clearance via apo E receptor (Gregg et al. 1986).

Approximately 40-65 % of people with AD have one or two apo E4 alleles compared to 20 % among the non AD population and more than 25 % of people carrying the apo E4 allele have AD (Lindsay et al. 2002). The presence of the apo E4 allele and risk of developing AD have been found to increase in an apo E4 dose dependent manner. For example, the presence of one apo E4 allele increases AD risk by 3 times and two apo E4 alleles increase AD risk by 8 times. Conversely, the presence of apo E2 alleles (E2/E2) decreases the risk by half and apo E2 can have a protective role (Corder et al. 1994) against AD. It is interesting to note people with an allele combination of E2/E4 are at similar risk to people with an E3/E3 allele combination suggesting that the detrimental effects of apo E4 could be nullified by the protective effects of apo E2. Clinical studies and animal studies show the presence of apo E4 results in a greater number of amyloid plaques (Sparks et al. 1996) in the brain compared to apo E3 (Carter et al. 2001, Gearing et al. 1996).

The presence of apo E4 is associated with changes in lipid profile and disorders in lipid metabolism. Familial type V hyperlipoproteinemia (Ghiselli et al. 1982), coronary heart disease (Wilson et al. 1996) and increased levels of CH

(hypercholesterolemia) (Utermann et al. 1984) are commonly associated with apo E4. Increased plasma CH, LDL-CH and apo B, and decreased HDL-CH and apo AI (Caramelli et al. 1999, Kuo et al. 1998) are reported in AD patients. The increased AD risk associated with the apo E4 allele could notionally be linked to a genetic influence of apo E4 on plasma lipid profile.

In addition, apo E4 can directly affect the development of amyloid plaques by influencing Aβ metabolism and/or other factors which influence plaque development; or indirectly, by decreasing choline acetyltransferase activity and nicotinic receptor binding sites (Poirier et al. 1995). The latter is implicated with treatment of AD by cholinesterase inhibitors but not involved in disease process (Poirier et al. 1995).

1.4.2 Role of lipoproteins and apolipoprotein E4 in beta-amyloid pathology

When Aβ bind with high affinity to lipoproteins and apolipoproteins they are referred to as lipidated Aβ, de-lipidated or free Aβ exists as well (Koudinov et al. 1994). Lipidation of Aβ has been suggested to play a role in the pathology of AD. Greater amounts of de-lipidated Aβ40 and Aβ42 were found in patients with MCI and AD subjects (Hanson et al. 2013, Matsubara et al. 1999). It is interesting to note that Alzheimer-prone, apo E4-containing subjects have the most delipidated or lipid free apo E, compared to E3 or E2 containing individuals (Hanson et al. 2013). In addition, C-terminal of lipidated apo E4 binds substantially more Aβ than apo E3 and apo E2 (Stratman et al. 2005). On the other hand, Tokuda et al. (2000) found greater association of lipid-bound apo E3 with Aβ compared to apo E4 subjects. In addition, the delipidation of apo E reduced its binding ability to Aβ by 5 to 10 fold. The reduced tendency for apo E to bind lipids may also affect its ability to sequester and clear Aβ, in turn, modulating the amount of free pathogenic Aβ. Apolipoprotein E4 individuals had the highest amount of de-lipidated or lipid-free apo E molecules compared to other isoforms (E4 >E3 >E2) (Hanson et al. 2013) which can impact on binding to Aβ. Takamura et al. (2011) found that dissociation of soluble Aβ from lipoproteins (de-lipidation) in CSF renders Aβ to a state that is more prone to oligomerization in the brain and soluble Aβ oligomers can directly cause synaptic loss (Hass and Selkoe 2007). Lipidation of Aβ by lipoproteins is pathologically

relevant as sequestration of Aβ can prevent deposition and thus protect the brain from toxicity.

1.4.3 Diet-genetic interaction of apolipoprotein E4

The synergistic effects of diet and lifestyle on risk for chronic diseases such as AD have been increasingly realized. Although the risk of apo E isoforms in AD has been established in western countries, an apo E4 paradox exists. In some populations the presence of apo E4 genetics does not correlate to increased prevalence of AD. Nigerians for example have a high incidence of apo E4 yet a low incidence of AD (Hendrie et al. 1996, Ogunniyi and Osuntokun 1991, Osuntokun et al. 1992). In a review article by Petot and Friedland (2004) it was shown that the association of apo E4 with AD was weaker for Africans, African-Americans, Arabs and Indians in comparison to Caucasians. Africans, for example were shown to have high prevalence of apo E4 and low prevalence of AD, African-Americans: a high prevalence of apo E4 and high prevalence of AD, Arabs: a low prevalence of apo E4 and high prevalence of AD, and finally East Indians with a moderate prevalence of apo E4 and low prevalence of AD (Farrer et al. 2003). Petot and Friedland (2004) attributed the paradox to the differences in total fat consumption across the population groups. When apo E4 individuals consume a high fat diet (>35 % kcal), the risk of developing AD is increased compared to a diet with less fat (<35 % kcal). However, the results from this study were based on food consumption questionnaire and results did not make discrepancy between types of fat consumed.

1.5 Dietary fat and Alzheimer's disease

1.5.1 Overview of dietary fat and Alzheimer's disease risk

Dietary fat is linked to both AD and other chronic diseases that are risk factors for AD. The prevalence of AD is positively influenced by increasing intake of calories and dietary fat. Caloric and dietary fat intake mid-life has been linked to developing AD in later life (Dosunmu et al. 2007). Excessive caloric intake and sedentary behaviour may directly influence AD risk (through unknown pathways) or indirectly increase risk as a consequence of secondary metabolic disorders or

diseases such as obesity (Brantley et al. 2005), cardiovascular disease and diabetes (Brookmeyer et al. 2007, Kivipelto et al. 2001).

In addition, there has been some indication of a link between ketogenic diets containing low carbohydrate and high fat levels with neurological diseases such as AD. Brownlow et al. (2013) used transgenic APP + PS1 mice and Tg4510 which were fed either ketogenic diet or control diet for 16 w. The ketogenic diet consists of 270 mg/kg of medium chain fatty acids as well as 70 g/kg of flaxseed oil and 60 g/kg of canola oil (compared to 0, 21 and 19 g/kg in control diets respectively). Carbohydrate was 62.2 g/kg in the control diet compared to 0.5 g/kg in the ketogenic diets and there was more fibre in the ketogenic diet (245.31 g/kg) compared to the control diet (40 g/kg). Following blood tests, results showed significantly greater levels of β-hydroxybutyrate and less glucose after 4 w of dietary regime. No changes in amyloid plaques or tau proteins were observed between control and ketogenic diets for all mice groups. In addition, there were no significant differences between the diets for open field test, radial arm water maze however there was a slight improvement of rotarod test for the ketogenic diets. The results from this study do not establish any specific link between the benefits of ketogenic diets in wild-type and transgenic mice with Alzheimer-like pathology.

The review by Stafstrom and Rho (2012) outlines the literature and provides an overview of ketogenic diets on a range of neurological disorders. This paper concludes that neurological disorders in general can be caused by an imbalance of energy metabolism, which can possibly be regulated by dietary therapy which favours a ketogenic diet. Interestingly, the author also notes that ketogenic diets can mimic the mechanisms and health benefits of caloric restriction by reducing glucose. The link between increased calorie intake and AD has been mentioned several times in the literature review chapter.

1.5.2 Effect of dietary fat on brain structure and function

Dietary nutrient intake is important for optimal brain function and evidence shows that SFAs affect neuronal function and may lead to neurological diseases such as dementia (Molteni et al. 2002, Sofrizzi et al. 2005) by influencing brain

structure, neuronal plasticity and memory (Greenwood and Winocur 2001, Molteni et al. 2002, Molteni et al. 2004, Winocur and Greenwood 1999).

Fats derived from dietary sources can be characterized as either SFAs or unsaturated fatty acids (UFAs) and are differentiated based on chain length and the presence of double bonds. Saturated fatty acids contain no double bonds whereas UFAs contains one or more double bonds. Unsaturated fatty acids containing one bond are known as MUFAs and those with more than one bond are referred to as PUFAs.

Diets high in SFAs have been reported to have detrimental effects on cognitive performance in humans, rats and mice (Sartorius et al. 2012, Winocur and Greenwood 1999) by altering neurobiology of the hippocampus (Davidson et al. 2012, Freeman and Granholm 2012) and hippocampus associated functions (review by Kanoski and Davidson 2011). Sartorius et al. (2012) uses milk fat and canola oil which as high in long chain SFA (predominantly palmitic acid 2.38 % and stearic acid 0.74%) and MUFA (Oleic acid 5.1 %), respectively. Male C57BL/6J mice were fed milk fat (3.3 % fat), canola oil (3.3 % fat) or standard chow (5 % fat – predominantly PUFA linoleic acid 3.51 %) for 8 weeks. Wincur and Greenwood (1999) also used diets which contained either 20 % (w/w) of soybean oil, or 18 % (w/w) beef tallow, 1% (w/w) soybean oil and 1 % (w/w) safflower oil or standard low fat diet containing 4.5 % (w/w) of fat. The diet containing beef tallow contains predominantly SFA (5.72 % palmitic acid and 4.1 % stearic acid), some MUFA (7.36% Oleic acid) and PUFA (2.02 % n-6) this dietary information was available in their previous publication by McGee and Greenwood (1990). The high energy diets used by Davidson et al. (2012) to feed the rats for 21 days included 17 % (w/w) as lard and 1.5 % (w/w) as safflower oil. Although the specific components of fatty acids were not provided in this study, lard contains 56-62 % UFA and 38-43 % SFA. The diet used by Freeman and Granholm (2012) uses 10 % hydrogenated coconut oil and 2 % cholesterol. Although coconut oil is known to contain high levels of SFA, the percentages of fatty acids were not provided and a search for custom diet “D2-AIN93” did not return any results on the manufacturer’s website (BP Biomedicals). Diets high in SFAs reduce levels of brain derived neurotrophic factor (BDNF) which protects neurons from toxicity and mice maintained on a SFA rich diet for 4 w performed poorer in the Morris Water Maze can be due to suppressive effects of

high SFA diet on BDNF (Molteni et al. 2002, Molteni et al. 2004, Wu et al. 2003). Studies by Molteni et al. (2002), Molteni et al. (2004) and Wu et al. (2003) have all used a similar diet to Davidson et al. (2012) using lard as their primary source of SFA. Diets high in SFAs promote oxidative stress by increasing free radical production which could be detrimental to brain function (Greenwood and Winocur 1990, Greenwood and Winocur 2001, Molteni et al. 2002, Kaplan and Greenwood 1998, Wu et al. 2003).

Conversely, MUFAs and PUFAs have been shown to be protective against (Hooijmans et al. 2009, Naqvi et al. 2011) or reverse cognitive decline (Morris 2004). In the study by Hooijimans et al. (2009), the PUFA diet contained 0.4% DHA, long chain PUFA with low n-6/n-3 ratio compared to SFA diet a with high SFA (type not specified) and low amount of long chain PUFA and high n-6/n-3 ratio. Naqvi et al. (2011) conducted a food frequency questionnaire on 441 women over the age of 60. These women completed both initial and follow-up cognitive assessments and had complete data. Data collected from the questionnaire were used to estimate the frequency and amount intake of each fatty acid from diet history in the past 3 years. This study found that high intake of MUFA was linked to reduced declines in cognitive performance. Longitudinal human studies show that PUFA and MUFA containing foods regulate neuronal transmission for healthy brain function (Solfrizzi et al. 2005) and are protective against neurodegeneration (Kalmijn et al. 1997, Morris 2004). There have also been important findings in experimental models that show rats fed PUFA perform better in Morris Water Maze assessment (Greenwood and Winocur 1990) and spatial tasks (Winocur and Greenwood 1999) compared to rats fed a high SFA diet or control diet. Saturated fat diet fed transgenic APP and wild-type mice show increased markers of astrocyte activiation (GFAP) expression, a marker of neuroinflammation (Julien et al. 2010).

It is important to consider other physiological or pathological consequences (such as glucose metabolism, cognitive decline and neurotoxicity) of high fat diets which can be independent of Aβ metabolism and AD pathology. Therefore, it is important to distinguish between direct and indirect influences of diet on AD pathology when assessing the literature and to focus specifically on Aβ metabolism and amyloidogenic pathways in relation to dietary fats.

1.5.3 Global pattern of Alzheimer's disease and dietary fat intake

Global variations in diet and lifestyle have been linked to the incidence and prevalence of AD (Grant 1998, Grant 2014). Geographically, AD is not uniform and there is a significantly lower prevalence in developing countries in comparison to developed countries (Chandra et al. 1998, Hendrie et al. 1995, Prince et al. 2004). The variation in AD incidence between countries has been linked to the cultural and dietary variations between countries (Grant 1998, Grant 2014). Countries with a high incidence of AD have higher average animal product, processed food and calorie intake than countries exhibiting a low incidence of AD. Moreover, increasing prevalence of AD in developing countries is thought to reflect increased urbanisation and a transition to western diet and lifestyle patterns.

In North and Latin America, the total percentage of the population with AD is higher (5.4 %) compared with Europe (4.4 % West > East), Africa (3.6 % North and Middle East), developed pacific regions (4.3 % Australia, Japan, South Korea and Singapore), developing western pacific regions (4.0 % China and Vietnam), and less developed regions (2.7 % Indonesia, Thailand and Sri Lanka; 1.9 % India and South Asia; 1.6 % South Africa) (Ferri et al. 2005).

Grant (2014) found that total caloric intake and intake of saturated and trans-fatty acids correlated with an increase in AD in Western countries compared to Asia and Africa (Kalmijn et al. 1997). Albanese et al. (2009) showed in middle and low-income countries (South America, China and India) that increased fish consumption was associated with a decreased risk of dementia and that dementia was associated with higher intake of meat. The author acknowledged that there was a potential lack of other relevant information such as the type of meat consumed, method of preparation and size of portions of meat.

The Honolulu-Asia Aging Study showed that when Japanese men who migrated from Japan to Hawaii had a much greater risk of developing AD (Havlik et al. 2000, Shadlen et al. 2000) which is consistent with research indicating a greater risk of AD among Japanese living in America compared to Japanese living in Japan (Graves et al. 1999). The cross-cultural study highlights the importance of environmental influences on the development of dementia and AD and that dietary

habits which increase the intake of fat and protein are linked to greater risk of developing AD (Shadlen et al. 2000).

1.5.4 Effects of different dietary fats on cognitive decline and Alzheimer's disease

Although general trends are suggestive that total fat and caloric intake are positively correlated with increasing prevalence of AD, epidemiological studies are consistent with the notion that AD can be a disease of lipid metabolism and is more significantly influenced by dietary fat type rather than quantity consumed.

1.5.4.1 Dietary saturated fat and cholesterol and Alzheimer's disease risk

1.5.4.1.1 Epidemiological evidence

An accumulation of epidemiological studies show that high dietary intake of SFA and CH is linked with increased prevalence of AD. Eskelinen et al. (2008) surveyed the participants of CAIDE and a random sample of 2000 individuals (aged 65-79). Total number of 1,449 participants completed the follow up tests (mean follow up time 4.9 years). Individuals diagnosed with dementia and those who did not complete the examination were excluded from the analysis, leaving a cohort of 1,341 individuals. Dietary intake was analysed by semi-frequency food questionnaire, identification of type and amount of fat intake was determined by questions which asked for amount of fat intake. In addition, the fatty acids types and quantities of SFA, MUFA and PUFA were calculated based on types of spreads and milk was ingested, as well as the fats used in cooking and baking. Results from this study showed that increased dietary SFA (from dairy) in midlife was associated with poorer cognitive function and MCI. Another study of 815 people aged 65 and over with 4 years follow-up showed that intake of SFA and trans-UFAs was positively associated with AD risk (Morris et al. 2003). Increased dietary intake of CH and high serum CH levels midlife has also been implicated in development of AD (Kivipelto et al. 2001, Kivipelto et al. 2002). In the Rotterdam Study of 5386 people over the age of 55, greater intake of SFAs and CH increased the risk of dementia after 2 years

follow-up (Kalmijn et al. 1997). Other large epidemiological studies show that high intake of SFAs and calories increase the risk of AD (Luchsinger et al. 2002).

The effect of dietary intake on the development of AD on the elderly partipants from the Chicago Health and Aging project was further examined by Morris et al. (2003). Out of the available cohort of 8501 subjects over the age of 65, 815 subjects were selected based on participation and cognitive state at the beginning of the experiment. Similar to the study by Eskelinen et al. (2008), this study has uses food-frequency questionnaire to evaluate the frequency and types of dietary fats ingested which allows analysis of SFA, PUFA and MUFA intake from dairy including spreads, chocolate; cooking/baking oil; fish oil supplement; eggs; meat and seafood. These choices presumably will give an accurate representation of frequency and types of fat ingested. Although limitations of these methods can include memory (questionnaire in the past year) and comprehension of the test, also test does not differentiate between the types of meat – for example, no option to select short cut or normal bacon (more fat) or grass fed or grain fed beef. These may impact on the type and content of fat ingested so some caution can be exercised whilst interpreting results.

Consistent with a positive association between elevated serum CH and AD risk, the use of CH lowering statins has been shown to decrease the prevalence of AD (Jick et al. 2000, Wolozin et al. 2000). However, the effectiveness of statins for use in dementia has been questioned. The meta-study by Ott et al. (2015) examines 25 articles (out of 5823 identified relevant articles from 2007 examining for the effective use of statins for dementia) and shows that statins can sometimes have adverse effects but only when the dose is used above the recommended limits. This study does not otherwise infer that the long term use of statins cause adverse effects on cognition. Statins can reduce levels of CH by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A reductase, the rate limiting step in CH biosynthesis pathway (Hamelin and Turgeon 1998). However, statins can also influence AD risk including suppression of the isoprenoid pathway (Bellosta et al. 2000), which has been implicated in AD (Cole and Vassar 2006). Statins are conventionally prescribed for vascular disease because high CH is a risk factor in atherosclerosis. However, the putative mechanisms as to how CH and statins can affect risk for AD are presently unresolved. Studies show that statins can impact on

membrane CH dynamics which can affect processing of membrane protein APP resulting in an aberrant increase in Aβ production (Eckert et al. 2005).

1.5.4.1.2 Animal models

Experiments in both wild type and transgenic animal models are supportive of the regulatory role of dietary fats in metabolism and cerebral deposition of Aβ protein. In adult New Zealand rabbits fed 2 % CH, both hypercholesterolemia and time-dependent increases in immunopositive cerebral amyloid deposits were noted after 4, 6 and 8 w of dietary intervention (Sparks et al. 1994). Intake of 5 % CH and 10 % fat in young APP Tg2576 transgenic mouse models for 7 w exhibited increased Aβ production and cerebral amyloidosis (Refolo et al. 2000). These results were replicated in another transgenic AD mice (TgAPPsw) fed high fat and CH chow for 7 or 10 months which induced significant increases in plasma lipids (VLDL and TGs) which correlated with increases in Aβ40 and Aβ42 plasma abundance (Shie et al. 2002). A similar experimental approach was explored by Ho et al. (2004) who used Tg2576 mice fed a high fat diet (60 % calorie intake from fat) compared with a low fat diet group (10 % calorie intake from fat). The study showed a 2-fold increase in amyloid 40 and 42 levels in the hippocampus, determined by sandwich ELISA in mice fed the high fat diet.

1.5.4.1.3 Cell models

Further evidence of the role of dietary fats in influencing intracellular APP processing has been established by a number of cell studies. In one study, the addition of SFAs; palmitic acid and stearic acid to a conditioned medium of APP transfected COS-7 cells significantly enhanced secretion of Aβ40 and Aβ42 whilst incubation with oleic acid and palmitoleic acid reduced secretions (Amtul et al. 2011a). In agreement with these results, Patil et al. (2006) also found direct stimulatory effects of palmitic acids and stearic acid on increased β-secretase processing of APP.

1.5.4.2 Dietary monounsaturated fat and Alzheimer's disease risk

1.5.4.2.1 Epidemiological evidence

An accumulating body of evidence suggests a protective and beneficial role of dietary MUFA in relation to AD risk. The classical Mediterranean diet is characterised by high levels of olive oil (predominantly MUFA), as well as fish, fruit, vegetables, legumes, cereals, moderate intake of dairy and lower intake of meat and SFAs. Although the Mediterranean diet can vary, the focus is on limited intake of processed foods and inclusion of nuts/seeds and whole grain cereals; moderate intake of dairy, eggs, fish and poultry, with lower intake of red meat; and moderate intake of red wine. The Mediterranean diet is 25-35 % dietary lipids and approximately 8-10 % can be calories from SFA from the above mentioned sources. However, not all Mediterranean diets are supportive of the beneficial diet in AD. Katsierdanis et al. (2013) examined a population of 557 elderly subjects with a food intake questionnaire and a cognitive assessment. Subjects showed no relationship between intakes of various dietary components (meat, vegetables, grains, sugar etc.) with mental performance. There was a non-significant increase in saturated fat intake among subjects (both men and women) who scored less in the cognitive tests. This cross-sectional study does not conclude if the Mediterranean diet per se contributes to the cognitive state but rather whether intake of specific components of the diet and lifestyle can influence cognitive decline among elderly individuals in the Mediterranean region.

Studies of dietary habits in the United States (Feart et al. 2009, Scarmeas et al. 2007), France (Tangney et al. 2011), Southern Italy (Solfrizzi et al. 1999) and more recently Australia (Gardener et al. 2012), suggest that increased intake of MUFAs can lower the risk of cognitive decline and presumably AD. Feart et al. (2009) studied in a sample of 9294 elderly subjects found that consuming Mediterranean diet over time was associated with slower rate of cognitive decline. Scarmeas et al. (2007) also conducted a longitudinal study in 192 individuals pre-diagnosed with AD found when subjects take in more "Mediterranean-type" foods, there were significantly linked to a reduced in rate of mortality. Tangney et al. (2011) also used a longitudinal study design; this study was performed on 3790 individuals which were assessed for cognition and food intake over a mean period of 7.6 years.

This study also concluded that adherence to Mediterranean diet was more associated with reduced rate of cognitive decline. In addition to oleic acid, other components of olive oil such as vitamin E (La Fata et al. 2014) and oleocanthal (Abuznait et al. 2013) have also been proposed to have beneficial effects on AD.

1.5.4.2.2 Animal models

Amtul et al. (2011) examined the effects of addition of oleic acid (MUFA) to a low fat chow diet in order to determine if oleic acid alone can influence Aβ levels in the brain. Transgenic mice (TgCRND8) mice were fed from 3 w to 20/21 w of age before they were sacrificed. The oleic acid diet contained the addition of oleic oil (2% w/w) to control low fat diet containing 1 % w/w soybean oil) and no cholesterol. Quantitative immunological analysis of brain tissue samples showed a significant decrease in Aβ42 levels in the hippocampus and lowered plaque in the hippocampus, amygdale and neurocortex compared to control low fat chow fed mice (Amtul et al. 2011b). The author concluded that this was linked to increased sAPPα and a decrease in β-secretase processing of APP in intracellular and membrane compartments which contain secretases.

1.5.4.2.3 Cell models

The addition of 0.1 µm oleic acid to transfected COS-7 cells transfected with APP695 decreased both Aβ40 and Aβ42 in conditioned medium compared to BSA controls (Amtul et al. 2010). Further investigation showed stimulatory effects of oleic acid on nonamyloidogenic pathway (increased soluble APPα by 50 %) and decreased amyloidogenic processing of APP (lower β- and γ-secreatase by 20 %). These results were comparable to animal studies where dietary administration of oleic acid resulted in a significant reduction in Aβ plaque in the neocortex, amygdale and hippocampus (Amtul et al. 2010).

1.5.4.3 Polyunsaturated fat and Alzheimer's disease risk

1.5.4.3.1 Omega-3 polyunsaturated fatty acids

There is increasing interest in the beneficial role of n-3 PUFA in chronic illnesses including AD. Consumption of 'oily fish' has been shown to be protective against cardiovascular disease, cognitive decline and AD (Barberger-Gateau et al. 2002, Freund-Levi et al. 2006, Kalmijn et al. 1997, Morris et al. 2003, Morris et al. 2006, Schaefer et al. 2006, van Gelder et al. 2007). Oily fish is a major source of n-3 PUFAs, mainly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), both of which are important for brain structure and function (Freund-Levi et al. 2006, Yurko-Mauro 2010). Docosahexaenoic acid is anti-oxidative and improves learning ability in animals (Calon et al. 2004, Gamoh et al. 2001, Lim and Suzuki 2000) and has also been shown to decrease Aβ secretion from neuronal cells (Lukiw et al. 2005). As the body can only synthesize small amounts of EPA and DHA from α-linolenic acid (Pawlosky et al. 2001), dietary intake of n-3 PUFA forms the major source. Despite findings that establish the benefits of PUFAs, some studies show no benefit of fish intake on dementia risk (Engelhart et al. 2002, Laurin et al. 2003). Studies show outcomes are also dependent on level of cognitive decline, presence of other chronic diseases, background dietary factors such as n6 PUFA, caloric intake and Devore et al. (2009) attributes the discrepancy between such findings as a result of different stages of AD progression.

1.5.4.3.2 Omega-6 polyunsaturated fatty acids

The main dietary source of n-6 fatty acids is vegetable oils but sources also include eggs, nuts, cereals and poultry. Linoleic acid and arachidonic acid are main types of n-6 and both can be converted to pro-inflammatory eicosanoids and prostaglandins. A Japanese study shows that increased n-6 PUFA intake is associated with increased risk of developing AD and vAD (Otsuka et al. 2002). To support this, Sanchez-Mejia et al. (2008) found greater n-6 and by-product of n-6 arachidonic acid was present in hippocampus of Alzheimer's hAPP mouse models indicating possible oxidative or inflammatory processes involved with presence of n-6 in brain. Indeed, cyclooxygenase and lipooxygenase enzymes which are

responsible for oxidation and conversion of n-6 PUFA to pro-inflammatory oxidative derivates have also been implicated in AD (Klegeris and McGeer 2002).

1.5.4.3.3 Ratio of omega-6/omega-3 fatty acids

It is unknown how the ratio of n-3 or n-6 can be related to dementia and AD. A cross sectional study in 304 elderly patients showed a relationship between a high n-6/n-3 ratio and cognitive decline (Gonzalez et al. 2010). Another study found increased n-6/n-3 ratio in AD cohorts compared to non-demented controls (Conquer et al. 2000). There are numerous reports of the benefits of n-3 PUFA (Cole et al. 2010, Fotuhi et al. 2009) and the pro-inflammatory factors of n-6 PUFA (Kuehl and Egan 1980). Although metabolic pathways of n-3 and n-6 are independent from each other, both n-3 and n-6 use the same enzymes and compete for cyclooxygenase, 5- lipoxygenase, elongase, delta-5-desaturase and delta-6-desaturase (Lewis et al. 1990). An imbalance in the n-3 and n-6 ratio, rather than total amount of n-3 and n-6 intake could therefore be of a greater relevance to AD pathology.

1.5.4.3.4 Animal models

Increases in dietary n-6 (safflower oil) in AD transgenic models increase synaptic loss (Calon et al. 2005, Calon et al. 2004, Cole et al. 2010). A direct relationship between dietary n-6/n-3 has been demonstrated in transgenic APP mice that showed that a DHA enriched diet (low n-6/n-3 ratio) compared to a DHA depleted diet (high n-6/n-3 ratio) reduced cortical Aβ burden by 70 % and reduced cerebral plaque burden by 40 % (Lim et al. 2005). A similar study conducted by Oksman et al. (2006) using transgenic APPswe/PS1dE9 mice on a diet with different n-6/n-3 ratios showed a significant reduction in Aβ42 levels in the hippocampus for diet with 1.4 n-6/n-3 ratio compared to their respective diets with higher proportions of n-6 relative to n-3 (ratios 8 and 70) (Oksman et al. 2006). The same study showed that 4 month 40 % SFA + 1 % CH feeding induced a non-significant increase in hippocampal Aβ40 and Aβ42 (Oksman et al. 2006). However, intervention with a diet containing 5 % DHA and 15 % SFA feeding reduced Aβ40 and Aβ42 in the hippocampus (Oksman et al. 2006). Another study in amyloid transgenic mice showed that both soluble and insoluble fractions of Aβ40 and Aβ42

levels increased but non-significantly with greater n-6/n-3 ratios (10.3 vs 2.8) compared to controls (Arsenault et al. 2011). Additive effects of PUFA with or without SFA have also been studied in transgenic amyloid mice models. A study by Julien et al. (2010) showed that a high ratio of n-6/n-3 (77) paired with low fat feeding (making up 5 % fat w/w) reduced cortical Aβ40 and Aβ42, whilst the same ratio with high SFAs derived from lard (35 % w/w) increased both soluble and insoluble Aβ after nine months feeding (Julien et al. 2010). The author concluded that dietary fat increased in cerebral amyloidosis was not linked to increase in total APP or APP processing products in brain and alteration of brain Aβ42/40 ratio was more likely (Julien et al. 2010).

1.5.4.3.5 Cell models

Cell studies relating to PUFAs and Aβ have mainly focused on the individual effects of n-6 or n-3 rather than the effects of changes to n-6/n-3 ratios. Amtul et al. (2010) showed that the addition of n-3 and n-6 PUFAs (EPA and AA) to APP transfected COS-7 cells resulted in an increase in secretion of Aβ40 and Aβ42 compared to DHA which showed a significant decrease in secretion in a concentration dependent manner. The author concluded that the concentration of DHA can modulate Aβ secretion via γ- and β-secretases (Amtul et al. 2010). Taken together, these studies suggest that intracellular changes in fat concentration and ratio can change production and secretion of APP.

1.6. Beta-amyloid production from lipogenic organs

1.6.1 Metabolism of exogenous and endogenously derived fats

Following the ingestion of a fatty meal and entrance into the stomach, bile emulsifies dietary fats in the small intestine. TGs are the major type of lipid absorbed from the diet. Pancreatic lipase hydrolyses TG to monoacylglycerol and fatty acids that can be readily absorbed by the small intestinal epithelial cells called enterocytes. Within enterocytes, assembly of nascent CMs occurs from the addition of TGs, CH, CH esters and phospholipids with apo B48 (Hussain et al. 2001, van Greevenbroek and de Bruin 1998). The enterocyte resynthesizes dietary derived

TG and also endogenous synthesized or new TG for obligatory incorporation into CM. A number of proteins are reported to associate with nascent CMs prior to secretion, including apo A-I, A-IV, apo J, apo D, apo E and small molecular weight proteins such as apo C-II. Nascent CMs are then transported via the Golgi apparatus to the basolateral membrane and secreted into mesenteric lymph and transported to the thoracic duct from which they enter the circulation via the subclavian vein. Circulating CMs interact with plasma HDL and receive apo CII and apo E. Apo CII on the surface of CMs can activate LPL which catalyse the hydrolysis of TGs from CMs for tissue use (predominantly liver, adipose and skeletal tissue). Hydrolysis by LPL results in the formation of smaller TG-poor and CH and apo E rich CM remnants. These apo E-rich CMs remnants participate in rapid clearance by the liver which is mediated by LDL-R and apo B100/E receptor-specific processes (Hussain et al. 1996). Please see other articles by Hussain (2014 review) for more detail regarding CM production.

Hepatocytes are the major liver cell type responsible for clearance of postprandial CMs. Dietary-derived substrate and endogenous biosynthesis of TG are secreted by hepatocytes as nascent VLDL. The physiochemical properties of VLDL are similar to CMs in that they are enriched in TG and comprise a large structural protein (apo B100). Like CMs, apo CII-activated hydrolysis of VLDL-TG by capillary lipases progressively hydrolyze TG, resulting in a lipid depleted VLDL remnant particles or intermediate density lipoprotein IDL. The remnant VLDL particles share clearance pathways with remnants of postprandial lipoproteins and to a large extent are cleared, however approximately half are hydrolyzed further by hepatic lipases resulting in CH-rich LDL. Circulating LDL interacts with LDL-receptors (via apo B) and is internalized via a classical endocytotic pathway involving lysosomal degradation.

In humans and some other species, CMs are distinguished from hepatically derived VLDL in that they contain a truncated variant of apo B100, a consequence of messenger ribonucleic acid editing. Enterocytic apo B48 is approximately half the amino acid length of apo B100, but serves the same principal function of providing structural integrity to relatively large macromolecules. Whilst metabolism of CMs and VLDL share similarities there are some notable differences. In comparison to VLDL,

the catabolic cascade is much quicker for CMs, a consequence of stereotactic interactions of apo E (the primary ligand for clearance) with apo B48.

1.6.2 Association between plasma triglycerides and Alzheimer's disease

There is accumulating evidence that disturbances in the metabolism of TRLs may be associated with increased risk for AD (Altman and Rutledge 2010). A cross-sectional study (Suryadevara et al. 2003) showed that probable AD patients had greater plasma levels of LDL and TG which was present irrespective of pre-existing vascular disease. A similar study (Sabbagh et al. 2004) of lipid levels in elderly population with probable AD shows increased TG levels in this group compared to healthy standard levels. Contrary to these findings, Presecki et al. (2011) reported a decrease in TG in subjects with MCI and a significant decrease in TG levels in subjects who performed worse in mental function exams. Whilst others found no significant association between fasting TG levels in AD and control subjects (Adunsky et al. 2002, Mamo et al. 2008, Merched et al. 2000, Reitz et al. 2004). Further evidence comes somewhat surprisingly from studies in amyloid transgenic mice. Burgess et al. (2006) reported that onset and progression of disease was associated with increased plasma Aβ and increased secretion into blood of TRL-Aβ.

1.6.3 The relationship between post-prandial lipid metabolism, beta-amyloid and Alzheimer's disease

A reason for discrepancy in findings could be the time of blood collection, as food intake and duration since last feed can impact on levels of TG. This information was not indicated by Adunsky et al. (2002), Reitz et al. (2004) and Sabbagh et al. (2004), where fasting was indicated, the duration was not mentioned by Suryadevara et al. (2003) and only Mamo et al. (2008) and Merched et al. (2000) stated respective overnight and 12 h fast prior to blood collection. The inconsistency in method makes it difficult to interpret plasma TG changes in AD. Disturbances in the metabolism of TRLs may be associated with increased risk for AD (Altman and Rutledge 2010). One study shows that postprandial lipoprotein metabolism is compromised in subjects with probable AD (Mamo et al. 2008). This study analysed both fasting (12 h fast) and post-absorptive (4 h fast after consuming meal containing 20 g fat, predominantly SFA) levels of apo B48 by immunoblot/enhanced

chemiluminescence methods. Results show a 3-fold increase in apo B48 levels compared to age-matched control subjects indicating greater plasma concentration of TRL in blood. Perhaps it would be more clinically relevant to test post-prandial level of TRLs in probable AD rather than fasting lipid levels since this study shows that probable AD subjects would have a disturbance in postprandial lipoprotein apo B48 concentration.

1.6.4 The distribution of beta-amyloid and plasma lipoproteins

Pathogenic relevance of TRLs in AD might be linked to the ability of lipoprotein to bind hydrophobic Aβ in plasma (James and Mamo 2005). A significant portion (95 %) of Aβ40 was found to be associated with lipoproteins in pooled AD plasma (Kuo et al. 1999). Inadvertently, this association makes detection of lipoprotein-bound Aβ more difficult for immunodetection due to lipid masking of epitopes (James and Mamo 2005). The plasma Aβ-lipoprotein distribution profile between probable AD, and control subjects were explored in one study. Despite showing non-significant changes in fasting plasma lipids, Mamo et al. (2008) reported that a significant amount of plasma Aβ was found with TRL (57-60 %) compared to LDL (12-29 %) and HDL (3.9-5.6 %). The association of plasma Aβ with lipoproteins, in particular TRLs indicates the possible role of Aβ in transport and metabolism of lipoproteins. Triglyceride-rich lipoproteins (density ≤ 1.019 g/mL) detected in this study includes CMs and VLDLs of intestine and liver origin. Although it is not possible to deduce the relative abundance of Aβ in CM or VLDL, a previous study found an avid association between Aβ and CMs (James et al. 2003) and the production and secretion of Aβ-lipoproteins within hepatocytes has also been reported (Koudinov et al. 1997).

Kontush (2004), in a review article proposes that Aβ could be an apo component of lipoproteins and the physiological role of Aβ is to regulate lipoprotein metabolism. As mentioned above, Aβ peptide has hydrophobic C-terminus which allows it to bind to hydrophobic substances such as lipids; the more pathologenic Aβ42 being more hydrophobic than the shorter Aβ40. Studies including those mentioned in this literature review indicate that Aβ binds with strong affinity to lipoproteins and apolipoproteins under physiological conditions. This review

underlines that Aβ, as a component of lipoproteins, could function in several ways including as an antioxidant, in synaptic activity and in regulation of lipid metabolism.

1.6.5 The association of apolipoprotein B with Alzheimer's disease

Apolipoprotein B is an apolipoprotein that is present on TRLs and is synthesized in the small intestine and the liver. Several clinical studies show that disturbances in apo B levels are detected in AD subjects. An increase in apo B levels is found in fasting and non-fasting states in AD patients compared to controls (Caramelli et al. 1999, Giubilei et al. 1990). In particular, apo B48, an obligatory marker for CMs, was shown to increase by 400 % in AD subjects compared to age-matched controls (Mamo et al. 2008). The relationship of Aβ with dietary fat and lipoprotein metabolism provides intriguing evidence that Aβ could be derived from lipogenic organs. The small intestine and liver are responsible for production of exogenous and endogenously derived fats respectively. Increased plasma TGs and apo B in AD subjects, coupled with specific increases of CMs in response to dietary fats suggests that Aβ pathology can be associated with post-ingestion kinetics of dietary fats.

1.6.6 Beta-amyloid production from lipogenic cells

Given that Aβ in plasma readily binds with avidity to lipoproteins derived from the small intestine or liver, the Aβ could also be produced and secreted from enterocytes and hepatocytes which synthesize apo B containing TRLs. A study using human liver carcinoma (HepG2) cells, which are common model to study human hepatocytes with great morphological and functional similarities, show presence of soluble Aβ40 within cell medium and cell lysates (Koudinov and Koudinova 1997). Analysis of conditioned cell medium revealed secretion of Aβ occurs under physiological conditions. Interestingly, secreted Aβ was recovered in complex 200-300 kDa with a diverse range of lipids (TG, CH, CH esters, phospholipids and apos) which are normally present on lipoproteins. In addition, intracellular Aβ associated with Golgi apparatus did not pass through the 30 kDa filter. Taken together, the author concluded that Aβ becomes incorporated into lipoproteins within the Golgi apparatus and Aβ is secreted in complex with lipoproteins (Koudinov and Koudinova 1997). This unique study put forward the

possibility that liver hepatocytes can contribute a large portion of plasma Aβ-lipoproteins. Although this study does not evaluate intestinal/enterocytic Aβ abundance, the enterocytes and hepatocytes share similar roles in production and secretion of apo B-containing lipoproteins and therefore, may also contribute to Aβ-lipoproteins in blood.

1.6.7 Plasma chylomicrons- beta-amyloid and Alzheimer's disease risk

The mechanism(s) underpinning the association of Western styled diets with increased risk for AD are not known. Like hepatocytes, some dietary fats may stimulate the production of CMs or CM-Aβ complex. Increased hepatic synthesis and secretion of VLDL-Aβ complex may synergistically exaggerate plasma lipoprotein-Aβ. Mamo et al. (2008) reported that plasma TG and CM concentrations were similar in fasted AD versus age matched controls. However, in response to ingestion of a mixed lipid meal, the post-absorptive plasma concentration of apo B48 (CMs) was remarkably four-fold higher in AD subjects compared to controls. The latter suggests dietary induced hyer-amyloidemia is not evident when fasted.

Transient postprandial elevations in the plasma concentration of soluble APP were reported in otherwise healthy subjects (Boyt et al. 1999) and in kinetic tracer studies, James et al. (2003) showed that Aβ may serve as an apolipoprotein of CMs. This study examined the possibility that Aβ can bind and follow the metabolic pathway of CMs throughout the transport and clearance of the particles. Using a synthetic model of Aβ bound to CM (synthetic Aβ was incorporated into an artificial lipoprotein molecule which is similar to chylomicrons [density = 1.006 g/ml]) and injected into the ear vein of new Zealand white rabbit, the particle was traced and identified in major organs – spleen, brain, liver, adipose tissue, muscle, bone marrow, lung and kidney. This study found iodine 125 radiolabelled Aβ took on similar metabolic pathway as cholesterol ester and transported primarily to fatty-rich tissues (adipose tissue [22.4 ng], liver [12.0 ng], bone marrow [8.0 ng] and muscle [2.6 ng]). In addition, the Aβ-CM like molecules was cleared at a strongly comparable pace as cholesterol esters over 30 min indicating that Aβ does not alter the kinetics of lipoprotein clearance. The association of Aβ with lipids supports the possibility that altered lipid metabolism, for example in cases where aberrations in postprandial lipoproteins, hyperlipidaemia or hypercholesterolaemia can affect

plasma Aβ homeostasis and therefore AD risk. This study also supports that this lipoprotein-Aβ complex can attach to vasculature in particular BBB via receptor-mediated processes.

1.6.8 Plasma beta-amyloid and postprandial lipoproteins contribute to cerebral pathology

Plasma lipid levels and also Aβ have been previously shown to be responsive to intake of dietary fats, although the causative reasons are currently not well understood. Postprandial lipemia refers to a period of time where changes in plasma lipids and lipoproteins occur after the ingestions of a high fat meal. This postprandial state is largely reflected by the increase in CMs, VLDL and remnant particles as well as a decrease in LDL and HDL (Vigna et al. 1999). This increase in CMs, VLDL and their respective remnants can play a role in the development of atherosclerosis by ineffective catabolism (Mamo et al. 1998) and the retention of these apo B-containing lipoproteins by endothelial cells (Proctor et al. 2002).

Numerous studies support increased dietary SFAs increase APP (Amtul et al. 2011a, Ho et al. 2004, Patil et al. 2006, Shie et al. 2002) processing which contributes to increased risk of developing AD (Julien et al. 2010, Koudinov and Koudinova 1997, Mamo et al. 2008, Olsson et al. 2003). Given that consumption of a fatty meal in humans can induce post-prandial lipidemia and high levels of apo B and TG, it is reasonable to assume that there is an increased amount of atherogenic CMs with Aβ and remnant particles present in plasma. It is possible to assume that an increase in plasma CMs can also mean increased levels of CM-Aβ in plasma as well under the postprandial state as there is clear evidence to suggest that AD patients could have aberrant postprandial lipoprotein metabolism including a build up of atherogenic remnant particles (Mamo et al. 1998). In addition, damage to the BBB and vasculature is frequently reported in AD patients and damaged BBB can mechanistically cause an increase in Aβ influx (refer to section 3). There is evidence that apo B, the obligatory apolipoprotein of intestinal and liver lipoproteins VLDLs colocalizes with cerebral amyloid plaques in humans and transgenic mouse models (Namba et al. 1992, Takechi et al. 2010b).

1.7 Section summary

The gradual “loss of mind” associated with AD is undoubtedly the most devastating consequence for individuals suffering with the disease. The Aetiology of the disease is unknown and there is presently no cure. Currently 300,000 people in Australia and 35 million people world-wide have AD and numbers are expected to triple by 2050. AD is a chronic disease of insidious nature followed by gradual and progressive loss of cognitive function which results in behavioral disturbances to episodic memory, language, executive function and visuospatial abilities. Abnormal deposits of insoluble amyloid protein and tau are visualised in post-mortem brain examinations of AD patients and are considered to be hallmark features of AD; however there is no explanation for the sporadic form of AD which constitutes about 99% of all cases.

An accumulating body of epidemiological, animal and cell research verifies the role of dietary fats in the pathogenesis of AD including a direct link between the amount and type of fat consumed and the production of Aβ, which has been identified as the main pathological protein in AD. Dietary fats can influence the risk of AD via regulation of the key steps of Aβ metabolism including production, transport/clearance and deposition.

Currently there is no direct evidence for the origin of cerebral plaque Aβ; however several key articles suggest plasma Aβ influx in sporadic AD could contribute to cerebral Aβ load. Dietary fat intake and CH can modulate this process possibly by altering BBB integrity, modulating the availability of Aβ chaperone proteins (including TRLs) and/or increasing the production of Aβ from APP.

Despite the use of compensatory treatments which offer some delay in the disease progression, AD progressively destroys cognitive function leading to eventual complete impairment and death of the affected person. Urgency is required for the better understanding of AD disease mechanisms in order to prevent the development of AD and delay the disease progression in AD affected persons. Current literature summarises that irreversible neurotoxicity in AD can be preventable through adherence to a healthy diet with selective fat intake.

CHAPTER 2

Chapter 2 – Intestinal Aβ expression and effects by high fat feeding

The content of this chapter is covered by article 1:

Galloway S, Jian L, Johnsen RD, Chew S, Mamo JCL. (2007) B-amyloid or its precursor protein is found in epithelial cells of the small intestine and is stimulated by high fat feeding. *J Nutr Biochem*. 18, 279-284

Objective addressed

Objective 2: To determine whether or not Aβ is expressed in small intestinal enterocytes and if this expression can be modified by a high fat and CH diet.

Hypothesis: Aβ is found in the small intestinal epithelial cells (enterocytes) that produce postprandial lipoproteins.

Article summary:

Cerebral deposition of Aβ peptide is causally associated early onset type AD, it is currently not known whether if Aβ plaques occur as a secondary event in late onset form of AD. The prevalent paradigm suggests that accumulation of Aβ in the brain initiates a cascade of events which leads to Aβ-induced synaptic toxicity and neuronal cell loss. Cerebral Aβ protein could be derived from the brain, the periphery, or a combination of both; the origin of Aβ remains unclear. Local and peripheral contributions are both possible considering that APP is ubiquitously expressed throughout the body; Aβ is produced under physiological conditions and is normally present in cells, interstitial fluid, plasma and CSF. In blood and CSF, Aβ associates with chaperone molecules and significant Aβ preferentially binds to lipoproteins. The presence of apo E, E4 is present in half of AD cases, and apo E is involved in transport of lipoproteins.

Epidemiological studies which show the risk of developing AD increases with high consumption of dietary fats, in particular SFAs and CH. This was additionally confirmed in animal models. High fat and CH feeding in animal models results in exacerbated cerebral Aβ plaque load compared to low fat feeding. There is intriguing evidence to suggest that Aβ can be involved in the metabolism of dietary regulated lipoprotein CMs. Chylomicrons are lipoproteins produced in the small intestinal epithelial cells and are secreted in response to high fat feeding. It is not known however whether or not the small intestine also produce Aβ. This study is aimed at investigating this possibility by using wild-type mice fed high fat CH diet. Beta-amyloid and/or APP were determined by semi-quantitative immunohistochemistry, the antibody AB5076 detects Aβ isoforms (includes 40 and 42) as well as APP. The expression of Aβ/APP was found within epithelial cells or enterocytes of the small intestine under low fat feeding. In addition, high fat CH feeding significantly increased enterocytic abundance of Aβ.

The findings are consistent with the possibility that Aβ could be a regulatory apolipoprotein of CMs. In addition, pathology of diet-induced cerebral amyloidosis can be linked to intestinal Aβ production and abundance. Chapter 2 will discuss details of this study.

Available online at www.sciencedirect.com
ScienceDirect
Journal of Nutritional Biochemistry 18 (2007) 279–284
Journal of Nutritional Biochemistry

β-Amyloid or its precursor protein is found in epithelial cells of the small intestine and is stimulated by high-fat feeding

Susan Galloway[a,b,c], Le Jian[b,c], Russell Johnsen[d,e], Stewart Chew[a], John C.L. Mamo[b,c,*]

[a]School of Biomedical Science, Curtin University of Technology, Perth, Western Australia 6102, Australia
[b]School of Public Health, Curtin University of Technology, Perth, Western Australia 6102, Australia
[c]Australian Technology Network, Center for Metabolic Fitness, Western Australia 6102, Australia
[d]Center for Neuromuscular and Neurological Disorders, University of Western Australia, Western Australia, Australia
[e]The Australian Neuromuscular Research Institute, Western Australia, Australia

Received 10 March 2006; received in revised form 9 June 2006; accepted 5 July 2006

Abstract

In Alzheimer's disease (AD), β-amyloid (Aβ) is deposited in extracellular matrices, initiating an inflammatory response and compromising cellular integrity. Epidemiological evidence and studies in animal models provide strong evidence that high-saturated-fat and/or cholesterol-rich diets exacerbate cerebral amyloidosis, although the mechanisms for this are unclear. Aβ contains hydrophobic domains and is normally bound to lipid-associated chaperone proteins. In previous studies, we have put forward the notion that Aβ is a regulatory component of postprandial lipoproteins (i.e., chylomicrons) and that aberrations in kinetics may be a contributing risk factor for AD. To explore this further, in this study, we utilized an immunohistochemical approach to determine if Aβ or its precursor protein is expressed in epithelial cells of the small intestine — the site of chylomicron biogenesis. Wild-type mice were fed a low-fat or a high-fat dietary regime and sacrificed, and their small intestines were isolated. We found that, in mice fed low-fat chow, substantial Aβ/precursor protein was found exclusively in absorptive epithelial cells of the small intestine. In contrast, no Aβ/precursor protein was found in epithelial cells when mice were fasted for 65 h. In addition, we found that a high-fat feeding regime strongly stimulates epithelial cell Aβ/precursor protein concentration. Our findings are consistent with the notion that Aβ may serve as a regulatory apolipoprotein of postprandial lipoproteins.

Keywords: Alzheimer's disease; Lipoproteins; Chylomicrons; Apoproteins; β-amyloid; Immunohistochemistry

1. Introduction

β-Amyloid (Aβ) is the predominant protein component of senile plaques found in subjects with Alzheimer's disease (AD) [1]. Current dogma suggests that deposition occurs when synthesis by neuronal cells exceeds the availability of chaperone transporters in the cerebrospinal fluid [2,3]. However, cerebrospinal fluid is an ultrafiltrate of plasma, raising the possibility that exogenous delivery of Aβ could exacerbate cerebral load [4,5]. Indeed, soluble forms of Aβ are found in plasma and within the junctions of epithelial cells that form the blood–brain barrier (BBB) [6], and the bidirectional movement of Aβ through the BBB has been described [7,8].

Sequestration of Aβ by chaperone proteins is pivotal to its continued solubility and underlies its tendency to otherwise cluster into complex oligomers [2,3,9]. A number of Aβ transport proteins have been described [10], but common to many of these is their normal coassociation with lipids in vivo. It is conceivable, that the physiological function of Aβ is related to the regulation of lipid metabolism, and consistent with this notion was the finding that Aβ enhanced the uptake of triglyceride-rich lipoproteins (TRLs) by fat-rich tissues, including brain tissues [11].

The kinetics of lipoproteins is dependent on apolipoproteins (apos) that serve as enzyme cofactors, or as ligands for receptors and extracellular matrices. Of particular interest is apoE because the E4 isoform is an established risk factor for

Abbreviations: Aβ, β-amyloid; AD, Alzheimer's disease; apo, apolipoprotein; APP, amyloid precursor protein; IHC, immunohistochemistry; TRL, triglyceride-rich lipoprotein; WT, wild type.

* Corresponding author. School of Public Health, Curtin University of Technology, Perth, Western Australia 6102, Australia. Tel.: +61 8 92667232; fax: +61 8 92662958.
E-mail address: J.Mamo@curtin.edu.au (J.C.L. Mamo).

0955-2863/$ – see front matter © 2007 Published by Elsevier Inc.
doi:10.1016/j.jnutbio.2006.07.003

AD [12]. ApoE tends to preferentially associate with plasma TRLs derived from the small intestine (chylomicrons) and the liver (very-low-density lipoprotein) [13,14]. Several lines of evidence suggest that Aβ is involved in the metabolism of dietary fats and that aberrations in postprandial lipemia might be a contributing factor for AD. There is a transient increase in the plasma concentration of amyloid precursor protein (APP), a surrogate marker of Aβ biosynthesis, following the ingestion of dietary fats [15]. Moreover, epidemiological studies have reported a positive association of fat intake with AD prevalence [16,17]. In animal studies, high-fat feeding induces cerebral amyloidosis, commensurate with dietary-induced hyperlipidemia and raised chylomicron concentration [18–20].

Aβ can be synthesized by the proteolytic cleavage of APP in the plasma membrane [14]. In addition, there is also significant intracellular abundance of Aβ associated with the rough endoplasmic reticulum (rER) and the Golgi apparatus [21,22]. The latter observations are consistent with the possibility that Aβ associates with primordial lipoproteins during biosynthesis. Given that lipoprotein synthesis is regulated by lipid–substrate availability, it is conceivable that fat-enriched diets exacerbate cerebral amyloidosis by also stimulating the synthesis and secretion of intestinal-derived chylomicron-Aβ. To explore this further, in this study, we utilized immunohistochemistry (IHC) to explore the putative effects of high-fat feeding on the intestinal expression of Aβ/APP in wild-type (WT) mice fed either a low-fat or a high-fat diet.

2. Animals and methods

2.1. Animals

The protocols used in this study were approved by the Curtin University Animal Experimentation and Ethics Committee (reference no. N 55-04). Six-week-old C57BL/6J mice were divided randomly into a low-fat group or a high-fat group. Low-fat mice were given cholesterol-free chow containing 4.0% (wt/wt) total fats (AIM93M; Specialty Feeds, Glen Forrest). Mice on the high-fat regime were given chow containing 1.0% (wt/wt) unsaturated fat, 16.0% total fat, 1% cholesterol and 0.5% cholate (SF00-245; Specialty Feeds). The digestible energy for low-fat and high-fat feeds was 15.1 and 18.7 MJ/kg, respectively, and feed was available ad libitum. Fasted mice were deprived of food for 65 h.

After 6 months of feeding, mice were anesthetized and exsanguinated. The small intestine was isolated, flushed with phosphate-buffered saline (PBS) and then fixed in 10% buffered formal saline. Transverse and longitudinal segments (2.0 mm thick) were taken at the same length of the proximal intestine from the duodenum–jejunum location and embedded in Paraplast. Serial sections (5 μm thick for histology and IHC) were cut, and all sections were mounted on silanized slides.

2.2. IHC

Tissue sections were deparaffinized and exposed to 3% hydrogen peroxide in methanol for 30 min to quench endogenous peroxidase activity. After washing, slides were incubated in blocking serum (20% goat serum) prior to incubation with rabbit antihuman Aβ(1–40/42) antiserum (AB5076; Chemicon, Temecula, CA), diluted to 1:800 with PBS containing 10% goat serum. After thorough washing with PBS, the slides were sequentially incubated with biotinylated goat antirabbit secondary antibody (E 0432; DAKO, Carpinteria, CA), followed by the addition of avidin–biotin–peroxidase complex (K 0377; DAKO). Positive immunostaining was established with liquid diaminobenzidine plus substrate chromogen kit (K 3467; DAKO). All slides were counterstained with Harris' hematoxylin.

The intensity of immunolabeling was quantified as previously described [23]. Labeling was considered adequate if it was moderate or intense, with adequately labeled positive controls and no labeling in negative controls. The total number of cells with different intensities of Aβ/APP staining was counted in each villus, and the data are expressed as a percentage of the total cells counted. Detection was determined every five sequential sections (i.e., at 25 μm). Four animals per group were investigated, with a minimum of two tissue blocks prepared for each. Mucosal epithelial cells were assessed from 25 randomly selected villi per slide, and a minimum of 100 cells in each villus were required to meet the granularity inclusion criteria.

Transgenic mice (Tg2576sw) expressing familial human APP695 [24] containing cerebral plaques were used for positive controls and to verify IHC procedures. For negative controls, Aβ-antiserum was replaced with either PBS or irrelevant serum (S20-100 ml; Chemicon). In addition, we verified that positive staining was abolished by the preabsorption of antiserum with free Aβ peptide (data not shown).

3. Results

3.1. Body weight and plasma lipids

We found no difference in weight gain during 6 months of (ad libitum) low-fat or high-fat diets; however, mice on high-fat diet were hypercholesterolemic (2.0±0.2 and 6.7±2.0 mmol/L, respectively).

3.2. Intestinal expression of Aβ/APP in mice fed low-fat and high-fat diets

In WT mice fed a low-fat and a high-fat diet, a positive Aβ/APP signal was observed throughout the epithelial cells of the intestinal mucosa. The pattern of Aβ/APP immunostaining was uniform throughout the villi and the crypts of Lieberkühn (Fig. 1B, C, E and F). Weak Aβ/APP staining was also present in the lamina propria; however, the majority of Aβ/APP staining was evident within the luminal

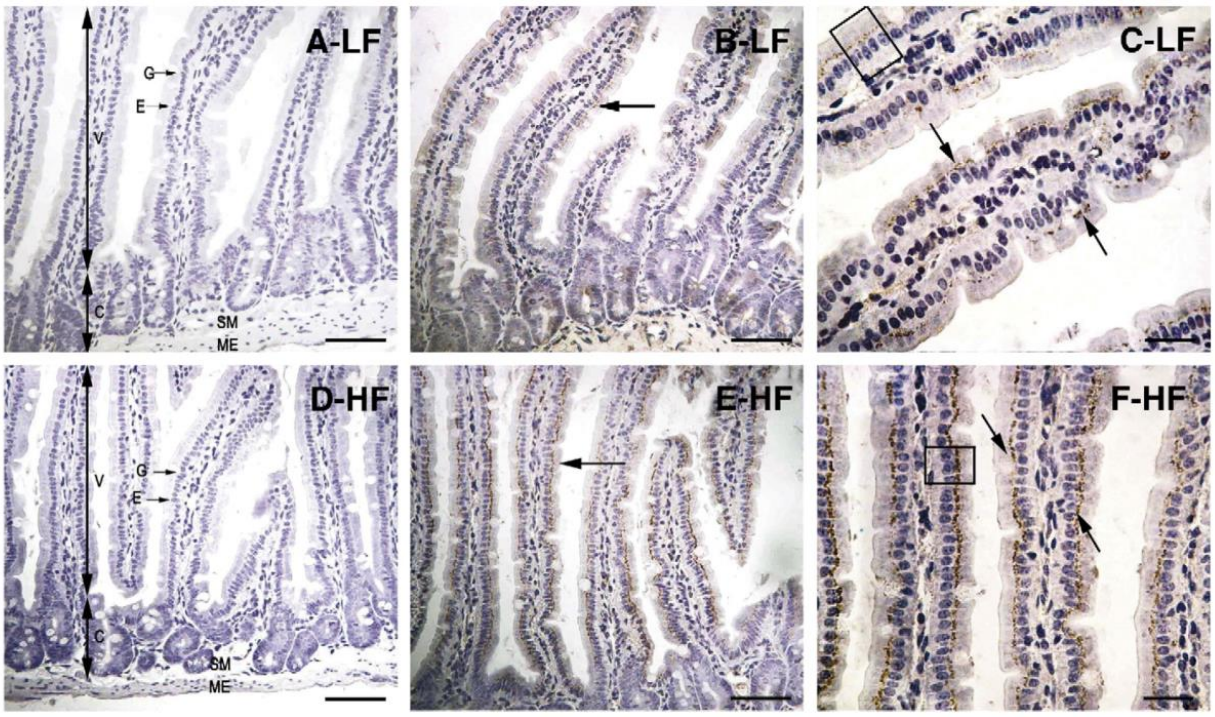


Fig. 1. Immunohistochemical detection of Aβ/APP in enterocytes of WT mice fed either a low-fat or a high-fat diet for 6 months. Intestinal sections at increasing magnifications show the pattern of Aβ/APP immunoreactivity (arrows in B, C, E and F) in the mucosal epithelium in mice fed a low-fat diet (LF) and in mice fed a high-fat diet (HF). Negative controls (irrelevant serum) are demonstrated for mice fed LF and mice fed HF in (A) and (D), respectively. Abundant Aβ/APP staining is observed within enterocytes of the small intestine and is enhanced for mice fed high-fat chow diet. Villi (V) and crypts (C) of the mucosa are clearly evident. Examples of enterocytes (E) and goblet cells (G) are indicated. Sm=submucosa; Me=muscularis externa. Scale bars=50 μm (A, B, D and E) and 20 μm (C and F).

villi. The distribution of Aβ/APP throughout the villus also increased with increasing proximity to the lumen. Cells with little or no evidence of Aβ include goblet cells, enteroendocrine cells, Paneth cells and undifferentiated stem cells. Within the epithelial cell cytoplasm, Aβ/APP exhibited granular morphology positioned superior to the nucleus. Immunolocalization of Aβ/APP in the perinuclear regions was consistent with the microvesicular regions of the cell that contain the rER and the Golgi apparatus (Fig. 1C and F).

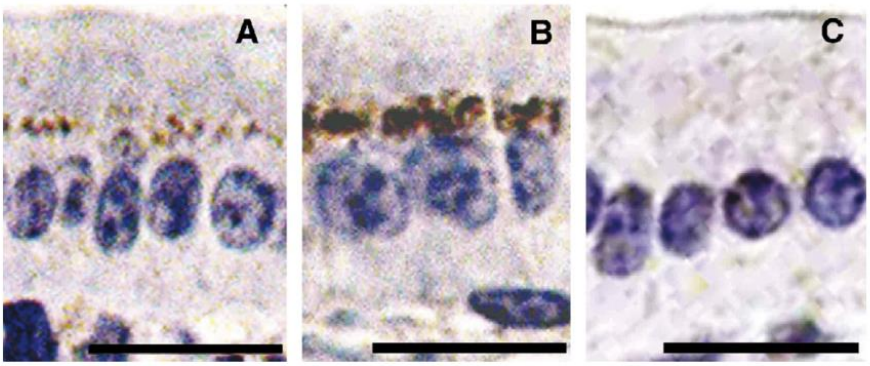


Fig. 2. Enterocytic expression of Aβ/APP in 6-month-old WT mice (C57BL/6J) fed a low-fat or a high-fat diet for 6 months or in mice fasted for 65 h. High magnifications of Aβ/APP immunostaining in enterocytes of the small intestine of mice fed a low-fat or a high-fat diet are shown in (A) and (B) (from Fig. 1), respectively. Substantial staining for Aβ/APP was detected within the perinuclear region of the cytoplasm, which was amplified by high-fat feeding. Note the increase in the size and density of Aβ/APP granules in absorptive cells in mice subjected to 6 months of high-fat diet (B) compared to low-fat diet (A). (C) Aβ/APP in epithelial cells of WT mice maintained on a high-fat diet but deprived of food for 65 h prior to sacrifice. Scale bar=5 μm.

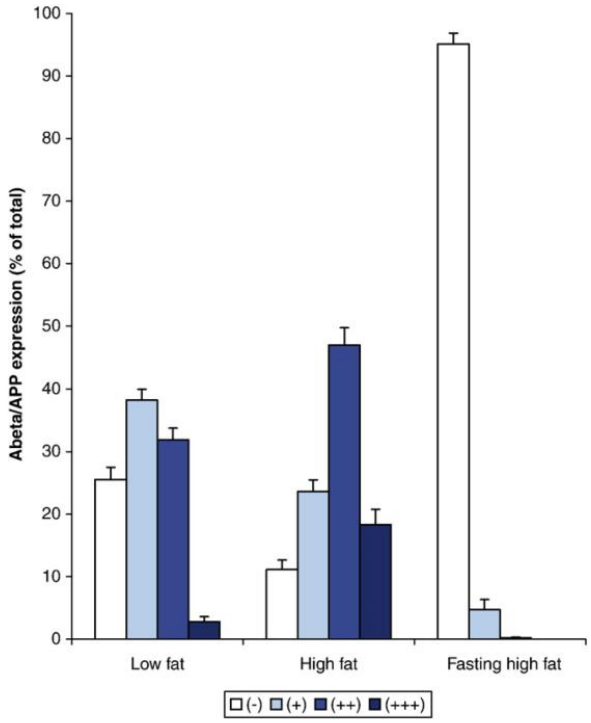


Fig. 3. Semiquantitative analyses of cellular Aβ/APP expressions in mice fed a high-fat or a low-fat diet and in mice fasted for 65 h (mice were initially given high-fat chow). Figure shows the proportion of cell numbers and the size of Aβ/APP expression between mice fed a high-fat diet and mice fed a low-fat diet, as well as animals fed a high-fat diet, which were deprived of food for 65 h prior to sacrifice. Each bar indicates the total average number of cells (y-axis), with difference intensity of Aβ/APP staining counted per mouse group (x-axis), and the data are presented as a percentage of the total cell count. Statistical significance in the high-fat group, relative to the low-fat group and the fasted-mice group, was determined for all group comparisons ($P<.01$). High-fat feeding significantly enhanced Aβ/APP intestinal expression compared to mice fed a low-fat diet. Additionally, 65 h of fasting significantly attenuated Aβ/APP immunostaining ($P<.05$). P values were calculated using Mann–Whitney test. (−) None, no immunolabeling; (+) weak, one to two granules; (++) moderate, three to four granules; (+++) high, larger three or more granules.

3.3. Chronic ingestion of cholesterol and saturated fats significantly enhances Aβ/APP immunostaining in absorptive columnar cells

In mice fed a high-fat diet, we found a strong positive expression of Aβ/APP in the small intestine that was qualitatively similar — but substantially enhanced — in comparison to the low-fat group (Fig. 2). The number of epithelial cells showing increasing signal intensity was determined. A greater proportion of cells stained positively in the high-fat group compared to the low-fat group (Fig. 3). In addition, a quantitative analysis of the degree of granularity found a greater proportion of cells in the high-fat group with moderate or higher signal staining for Aβ/APP compared to the weaker signal in low-fat mice. In contrast, food deprivation for 65 h in mice fed a high-fat diet abolished epithelial cell Aβ staining (Fig. 2C).

4. Discussion

This study utilized an immunohistochemical technique to explore if Aβ/APP is expressed in the absorptive epithelial cells of the small intestine. Aβ-antiserum was specific for Aβ/APP based on the staining of amyloid-rich cerebral plaques in positive control tissues, and this was abolished by competition with exogenous soluble Aβ.

In WT mice, Aβ/APP immunoreactivity was visible in columnar absorptive epithelial cells. The significant abundance of Aβ/APP within the perinuclear region is consistent with previous findings in cell cultures demonstrating the intracellular synthesis of Aβ from the rER and the Golgi apparatus [21,22,25–27]. The cytoplasmic perinuclear distribution of Aβ/APP is consistent with the site of chylomicron lipid pools within enterocytes [28]. The qualitative patterns of Aβ/APP distribution were similar for mice fed the high-fat diet and mice fed the low-fat diet. However, we found a significantly greater intestinal epithelial expression of Aβ/APP in mice given the high-fat diet ($P<.001$). Coupled with recent findings that Aβ binds avidly with chylomicrons [11], our observations support the concept that chronic high-fat feeding increases intestinal Aβ association with chylomicrons and that Aβ regulates dietary fat metabolism. Staining for Aβ/APP was mostly observed in mature enterocytes, which have the greatest rates of chylomicron production [29]. Biosynthesis of chylomicrons occurs in a multistep process that requires the progressive lipidation of apos [30,31], and Aβ is an amphipathic protein that avidly associates with large negatively charged hydrophobic lipids [32].

Fat feeding stimulates chylomicron synthesis [30,33], and it is possible that greater enterocytic staining for Aβ/APP is a reflection of increased rates of chylomicron-Aβ production. Alternatively, Aβ/APP may accumulate within the enterocyte during the postabsorptive state following an ingestion of fatty foods [30]. Once in the plasma, chylomicrons are rapidly hydrolyzed by endothelial lipases to produce a cholesterol-enriched and apoE-enriched remnant lipoprotein, which is normally cleared rapidly by receptor processes [34]. However, chronic high-fat and cholesterol feeding results in an accumulation of remnant lipoproteins because of depressed levels of high-affinity uptake pathways [35]. Mice that were given chow enriched with saturated fats and cholesterol were dyslipidemic and, by extension, may also have had greater circulating levels of plasma Aβ.

We have previously reported that Aβ enhances chylomicron tissue uptake by fat-rich tissues, including brain tissues [11]. Following the ingestion of fats, higher concentrations of postprandial chylomicron Aβ may be present in the plasma, and these may remain elevated with habitual intake of foods rich in saturated fats or cholesterol [33]. Increasing plasma levels of chylomicron-Aβ may adversely influence cerebral Aβ homeostasis, resulting in accelerated deposition. Indeed, apoB100 and apoB48, equivocal markers of hepatic and

intestinal lipoproteins, respectively, have been reported in cerebrospinal fluid [11,36–38].

This study demonstrates an enhanced Aβ/APP expression within the enterocytes of the mouse small intestine in response to saturated fat/cholesterol feeding. We propose that the intracellular synthesis of Aβ is associated with nascent chylomicron biogenesis via the posttranslational addition of lipids. Further studies are required to determine if Aβ biogenesis is coupled to the synthesis and secretion of chylomicrons and to explore how this phenomenon can be regulated by specific macronutrients and/or pharmacotherapies.

References

[1] Masters CL, Multhaup G, Simms G, Pottgiesser J, Martins RN, Beyreuther K. Neuronal origin of a cerebral amyloid: neurofibrillary tangles of Alzheimer's disease contain the same protein as the amyloid of plaque cores and blood vessels. EMBO J 1985;4:2757–63.

[2] Goldgaber D, Schwarzman AI, Bhasin R, Gregori L, Schmechel D, Saunders AM, et al. Sequestration of amyloid beta-peptide. Ann N Y Acad Sci 1993;695:139–43.

[3] Wisniewski T, Ghiso J, Frangione B. Alzheimer's disease and soluble A beta. Neurobiol Aging 1994;15(2):143–52.

[4] Seubert P, Vigo-Pelfrey C, Esch F, Lee M, Dovey H, Davis D, et al. Isolation and quantification of soluble Alzheimer's beta-peptide from biological fluids. Nature 1992;359:325–7.

[5] Shoji M, Golde TE, Ghiso J, Cheung TT, Estus S, Shaffer LM, et al. Production of the Alzheimer amyloid beta protein by normal proteolytic processing. Science 1992;258:126–9.

[6] Glenner GG, Wong CW. Alzheimer's disease and Down's syndrome: sharing of a unique cerebrovascular amyloid fibril protein. Biochem Biophys Res Commun 1984;122:1131–5.

[7] Zlokovic BV, Ghiso J, Mackic JB, McComb JG, Weiss MH, Frangione B. Blood–brain barrier transport of circulating Alzheimer's amyloid beta. Biochem Biophys Res Commun 1993;197:1034–40.

[8] Mackic JB, Bading J, Ghiso J, Walker L, Wisniewski T, Frangione B, et al. Circulating amyloid-beta peptide crosses the blood–brain barrier in aged monkeys and contributes to Alzheimer's disease lesions. Vascul Pharmacol 2002;38:303–13.

[9] Ghiso J, Matsubara E, Koudinov A, Choi-Miura NH, Tomita M, Wisniewski T, et al. The cerebrospinal-fluid soluble form of Alzheimer's amyloid beta is complexed to SP-40,40 (apolipoprotein J), an inhibitor of the complement membrane-attack complex. Biochem J 1993;293:27–30.

[10] Biere AL, Ostaszewski B, Stimson ER, Hyman BT, Maggio JE, Selkoe DJ. Amyloid beta-peptide is transported on lipoproteins and albumin in human plasma. J Biol Chem 1996;271:32916–22.

[11] James AP, Pal S, Gennat HC, Vine DF, Mamo JC. The incorporation and metabolism of amyloid-beta into chylomicron-like lipid emulsions. J Alzheimer's Dis 2003;5:179–88.

[12] Strittmatter WJ, Weisgraber KH, Huang DY, Dong LM, Salvesen GS, Pericak-Vance M, et al. Binding of human apolipoprotein E to synthetic amyloid beta peptide: isoform-specific effects and implications for late-onset Alzheimer disease. Proc Natl Acad Sci U S A 1993;90:8098–102.

[13] Mahley RW. Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. Science 1988;240:622–30.

[14] Saito H, Dhanasekaran P, Baldwin F, Weisgraber KH, Phillips MC, Lund-Katz S. Effects of polymorphism on the lipid interaction of human apolipoprotein E. J Biol Chem 2003;278:40723–9.

[15] Boyt AA, Taddei K, Hallmayer J, Mamo J, Helmerhorst E, Gandy SE, et al. Relationship between lipid metabolism and amyloid precursor protein and apolipoprotein E. Alzheimer's Rep 1999;2:339–46.

[16] Kalmijn S, Launer LJ, Ott A, Witteman JC, Hofman A, Breteler MM. Dietary fat intake and the risk of incident dementia in the Rotterdam Study. Ann Neurol 1997;42:776–82.

[17] Solfrizzi V, D'Introno A, Colacicco AM, Capurso C, Del Parigi A, Capurso S, et al. Dietary fatty acids intake: possible role in cognitive decline and dementia. Exp Gerontol 2005;40:257–70.

[18] Sparks DL, Scheff SW, Hunsaker III JC, Liu H, Landers T, Gross DR. Induction of Alzheimer-like beta-amyloid immunoreactivity in the brains of rabbits with dietary cholesterol. Exp Neurol 1994;126:88–94.

[19] Refolo LM, Malester B, LaFrancois J, Bryant-Thomas T, Wang R, Tint GS, et al. Hypercholesterolemia accelerates the Alzheimer's amyloid pathology in a transgenic mouse model. Neurobiol Dis 2000;7:321–31.

[20] Shie FS, Jin LW, Cook DG, Leverenz JB, LeBoeuf RC. Diet-induced hypercholesterolemia enhances brain A beta accumulation in transgenic mice. Neuroreport 2002;13:455–9.

[21] Cook DG, Forman MS, Sung JC, Leight S, Kolson DL, Iwatsubo T, et al. Alzheimer's A beta(1–42) is generated in the endoplasmic reticulum/intermediate compartment of NT2N cells. Nat Med 1997;3:1021–3.

[22] Wild-Bode C, Yamazaki T, Capell A, Leimer U, Steiner H, Ihara Y, et al. Intracellular generation and accumulation of amyloid beta-peptide terminating at amino acid 42. J Biol Chem 1997;272:16085–8.

[23] Mikaelian I, Nanney LB, Parman KS, Kusewitt DF, Ward JM, Naf D, et al. Antibodies that label paraffin-embedded mouse tissues: a collaborative endeavor. Toxicol Pathol 2004;32:181–91.

[24] Hsiao KK, Borchelt DR, Olson K, Johannsdottir R, Kitt C, Yunis W, et al. Age-related CNS disorder and early death in transgenic FVB/N mice overexpressing Alzheimer amyloid precursor proteins. Neuron 1995;15:1203–18.

[25] Tomita T, Tokuhiro S, Hashimoto T, Aiba K, Saido TC, Maruyama K, et al. Molecular dissection of domains in mutant presenilin 2 that mediate overproduction of amyloidogenic forms of amyloid beta peptides. Inability of truncated forms of PS2 with familial Alzheimer's disease mutation to increase secretion of Abeta42. J Biol Chem 1998;273:21153–60.

[26] Petanceska SS, Seeger M, Checler F, Gandy S. Mutant presenilin 1 increases the levels of Alzheimer amyloid beta-peptide Abeta42 in late compartments of the constitutive secretory pathway. J Neurochem 2000;74:1878–84.

[27] Iwata H, Tomita T, Maruyama K, Iwatsubo T. Subcellular compartment and molecular subdomain of beta-amyloid precursor protein relevant to the Abeta 42-promoting effects of Alzheimer mutant presenilin 2. J Biol Chem 2001;276:21678–85.

[28] Ross MH, Kaye GI, Pawlina W, editors. Digestive system: II. Esophagus and gastrointestinal tract. 16.41 Histology: a text and atlas. Philadelphia: Lippincott Williams and Wilkins; 2003. p. 495.

[29] Pageot LP, Perreault N, Basora N, Francoeur C, Magny P, Beaulieu JF. Human cell models to study small intestinal functions: recapitulation of the crypt–villus axis. Microsc Res Tech 2000;49:394–406.

[30] van Greevenbroek MM, de Bruin TW. Chylomicron synthesis by intestinal cells in vitro and in vivo. Atherosclerosis 1998;141(Suppl 1):S9–S16.

[31] Hussain MM, Kedees MH, Singh K, Athar H, Jamali NZ. Signposts in the assembly of chylomicrons. Front Biosci 2001;6:D320–31.

[32] Shao H, Jao S-C, Ma K, Zagoriski MG. Solution structures of Michelle-bound amyloid beta1–40 and beta1–42 peptides of Alzheimer's disease. J Mol Biol 1999;285:755–73.

[33] Luchoomun J, Hussain MM. Assembly and secretion of chylomicrons by differentiated Caco-2 cells. Nascent triglycerides and preformed phospholipids are preferentially used for lipoprotein assembly. J Biol Chem 1999;274:19565–72.

[34] Hultin M, Olivecrona T. Conversion of chylomicrons into remnants. Atherosclerosis 1998;141:25–9.

[35] Mamo JC, Watts GF, Barrett PH, Smith D, James AP, Pal S. Postprandial dyslipidemia in men with visceral obesity: an effect of

reduced LDL receptor expression? Am J Physiol Endocrinol Metab 2001;281:E626–32.
[36] Anderson GJ, Tso PS, Connor WE. Incorporation of chylomicron fatty acids into the developing rat brain. J Clin Invest 1994;93:2764–7.
[37] Kou YM, Emmerling MR, Bisgaier CL, Essenburg AD, Lampert HC, Drumm D, et al. Elevated low density lipoprotein in Alzheimer's disease correlates with brain Abeta 1–42 levels. Biochem Biophys Res Commun 1998;252:711–5.
[38] Osman I, Gaillard O, Meillet D, Bordas-Fonfrede M, Gervais A, Schuller E, et al. A sensitive time-resolved immunofluorometric assay for the measurement of apolipoprotein B in cerebrospinal fluid. Application to multiple sclerosis and other neurological diseases. Eur J Clin Chem Clin Biochem 1995;33:53–8.

CHAPTER 3

Chapter 3 – Effect of apo E knockout on intestinal Aβ expression

The content of this chapter is covered by article 2:

Galloway S, Pallebage-Gamarallage MM, Takechi R, Jian L, Johnsen RD, Dhaliwal SS, Mamo JC. (2008) Synergistic effects of high fat feeding and apolipoprotein E deletion on enterocytic amyloid beta peptide abundance. *Lipids Health Dis*. 7, 15.

Objective addressed:

Objective 3: To determine if apo E deletion in the presence of high fat feeding would have an effect on intestinal Aβ in wild-type and apo E gene knockout mice.

Hypothesis: Apolipoprotein E can modulate enterocytic Aβ expression and this can be influenced by changes in dietary fat content.

Article summary:

We have previously determined the presence of Aβ within the small intestinal absorptive epithelial cells with specific immunological methods. Our observations showed that whilst high fat (HF) feeding increased abundance of enterocyte Aβ dramatically, fasting resulted in abolishment of Aβ expression. An allelic variation of apo apo E, apo E4, increases the risk of developing AD by modulating production, transport/solubility and clearance of Aβ. Apolipoprotein E is involved in redistribution of CH in the body and in the presence of apo E4, there is an accumulation of CH. High dietary fat-induced hypercholesterolemia in wild-type (WT) and transgenic AD animals resulted in greater levels of cerebral Aβ compared to low fat (LF) chow fed controls.

To investigate the role of apo E in intestinal Aβ abundance, genetically modified apo E null mice and control WT animals were used. The possible

synergistic effect of HF feeding was determined by feeding apo E knockout (KO) mice on HF and CH diets. Immunohistochemistry was used to determine semi-quantitative abundance of Aβ. We found increased intestinal Aβ under HF feeding compared to LF fed apo E KO mice. In addition, plasma CH was significantly enhanced in apo E KO mice compared to WT controls. Intestinal villi height increased with HF feeding in WT mice, whilst remained unchanged in apo E KO mice.

The mechanism by which HF feeding increases enterocytic Aβ abundance in apo E KO mice is unclear. However, some studies show apo E can interact with γ-secretase activity and modulate proteolysis of Aβ from APP. High fat feeding increases absorption of lipids and synthesis of CMs and Aβ via greater availability of lipid substrate.

Lipids in Health and Disease

BioMed Central

Research

Open Access

Synergistic effects of high fat feeding and apolipoprotein E deletion on enterocytic amyloid-beta abundance

Susan Galloway[1], Menuka MS Pallebage-Gamarallage[1], Ryusuke Takechi[1], Le Jian[1], Russell D Johnsen[2,3], Satvinder S Dhaliwal[1] and John CL Mamo*[1]

Address: [1]School of Public Health and Australian Technology Network (ATN), Centre for Metabolic Fitness, Curtin University of Technology, Perth, Western Australia, [2]Australian Neuromuscular Research Institute, QEII Medical Centre, Perth, Western Australia and [3]Centre for Neuromuscular and Neurological Disorders, University of Western Australia

Email: Susan Galloway - susan.galloway@postgrad.curtin.edu.au; Menuka MS Pallebage-Gamarallage - mmenuka@hotmail.com; Ryusuke Takechi - ryusuke.takechi@postgrad.curtin.edu.au; Le Jian - L.Jian@Curtin.edu.au; Russell D Johnsen - rjohnsen@cyllene.uwa.edu.au; Satvinder S Dhaliwal - S.Dhaliwal@Curtin.edu.au; John CL Mamo* - J.Mamo@Curtin.edu.au

* Corresponding author

Published: 22 April 2008

Lipids in Health and Disease 2008, **7**:15 doi:10.1186/1476-511X-7-15

Received: 29 January 2008
Accepted: 22 April 2008

This article is available from: http://www.lipidworld.com/content/7/1/15

Abstract

Background: Amyloid-β (Aβ), a key protein found in amyloid plaques of subjects with Alzheimer's disease is expressed in the absorptive epithelial cells of the small intestine. Ingestion of saturated fat significantly enhances enterocytic Aβ abundance whereas fasting abolishes expression. Apolipoprotein (apo) E has been shown to directly modulate Aβ biogenesis in liver and neuronal cells but it's effect in enterocytes is not known. In addition, apo E modulates villi length, which may indirectly modulate Aβ as a consequence of differences in lipid absorption. This study compared Aβ abundance and villi length in wild-type (WT) and apo E knockout (KO) mice maintained on either a low-fat or high-fat diet. Wild-type C57BL/6J and apo E KO mice were randomised for six-months to a diet containing either 4% (w/w) unsaturated fats, or chow comprising 16% saturated fats and 1% cholesterol. Quantitative immunohistochemistry was used to assess Aβ abundance in small intestinal enterocytes. Apo E KO mice given the low-fat diet had similar enterocytic Aβ abundance compared to WT controls.

Results: The saturated fat diet substantially increased enterocytic Aβ in WT and in apo E KO mice, however the effect was greater in the latter. Villi height was significantly greater in apo E KO mice than for WT controls when given the low-fat diet. However, WT mice had comparable villi length to apo E KO when fed the saturated fat and cholesterol enriched diet. There was no effect of the high-fat diet on villi length in apo E KO mice.

Conclusion: The findings of this study are consistent with the notion that lipid substrate availability modulates enterocytic Aβ. Apo E may influence enterocytic lipid availability by modulating absorptive capacity.

Background

Net concentration of cerebral Aβ is determined by the presence of apolipoprotein (apo) E with a dose dependent gene effect of apo E -/- < apo E -/+ < apo E +/+ on hippocampal senile plaques [1,2]. Animals and cell culture studies show that apo E regulates the production, transport,

clearance and solubility of Aβ [1-8]. Apolipoprotein E may modulate cerebral Aβ homeostasis by regulating cerebral Aβ efflux via the low-density-lipoprotein-receptor-related protein (LRP), relative to the influx of Aβ via transporters such as the receptor for advanced-glycation-end-products (RAGE) [9]. In addition, apo E can also directly influence Aβ biogenesis via regulation of α- and β-secretases activity [10], or indirectly, by influencing the intracellular pool of regulating lipids [11].

Apolipoprotein E critically regulates cholesterol metabolism and lipid homeostasis. The apo E protein is the primary receptor ligand for dietary-derived lipoproteins synthesized by the small intestine (chylomicrons) and triglyceride-rich lipoproteins (very-low-density lipoproteins (VLDL)), synthesized from liver [12]. Several lines of evidence support a link between aberrations in lipid metabolism and AD risk [7,11,13]. Epidemiological and clinical studies suggest that a high intake of saturated fat and/or cholesterol accelerate onset and progression of AD, whereas some polyunsaturated fatty acids may be protective [13-17]. Moreover, strong evidence of a causal relationship between dietary fats and AD comes from feeding studies in mice or rabbits. Animals given saturated-fat diets show significant immuno-detectable cerebral Aβ burden [18-20], although the mechanisms by which this occurs are presently unclear.

Our laboratory recently reported that absorptive epithelial cells of the small intestine secrete Aβ associated with dietary-derived lipoproteins (chylomicrons) [21]. A diet enriched in saturated fats and cholesterol was found to markedly increase enterocytic Aβ, whereas fasting completely abolished Aβ production. Chronic ingestion of saturated-fat may lead to sustained elevations in blood of lipoprotein-bound Aβ, because of overproduction and thereafter, reduced clearance from blood. Moreover, recent studies suggest that exaggerated exposure to circulating Aβ may compromise blood-brain-barrier integrity and exacerbate cerebral amyloidosis [22]. In normal subjects, approximately 60% of lipoprotein-bound plasma Aβ is associated with the triglyceride-rich-lipoproteins (TRL's) and in subjects with AD, post-absorptive accumulation of chylomicrons has been identified [23].

Apolipoprotein E is pivotal for the interaction of TRL with high affinity clearance pathways [12] including the low-density-lipoprotein-receptor (LDL-r) and LRP and will therefore significantly influence plasma lipoprotein-Aβ concentration and kinetics. However, apo E may also influence plasma Aβ homeostasis by modulating synthesis and secretion of the lipoprotein-Aβ complex from either the intestine and/or liver. To explore this concept further, in this study we compared enterocytic Aβ homeostasis in wild-type mice versus animals devoid of apo E (apo E knockouts). Mice were given either a low-fat, or high saturated-fat diet to explore synergistic effects. We find that apo E modulates intestinal morphology in a manner which may influence lipid absorptive capacity and has a synergistic effect with dietary fats on enterocytic Aβ homeostasis.

Results

High-fat feeding induced hypercholesterolemia in apo E KO mice

Apo E KO mice given low-fat chow had significantly elevated plasma cholesterol compared to WT mice on the low-fat diet (table 1), however plasma triglycerides were not significantly affected because of the gene deletion. In WT mice the high saturated fat diet had no significant affect on plasma cholesterol or triglycerides (table 1). However, in apo E KO mice hypercholesterolemia was substantially exacerbated and some two-fold greater than the apo E KO mice given low-fat chow. All groups of mice gained weight during the intervention and there was no significant difference between treatment groups (data not shown).

Immunolocalisation of Aβ in the small intestine of apolipoprotein E KO mice: synergistic effects of high fat feeding

For all groups of mice, Aβ immunostaining was demonstrated within the perinuclear region of absorptive columnar epithelial cells of the small intestine mucosa (insert, figure 1). With low-fat feeding, WT and apo E KO mice

Table 1: Plasma lipids in wild-type and apolipoprotein E knockout mice fed low and high fat diets

Diet	Gene	Cholesterol (mM) mean ± S.E.M	Triglyceride (mM) mean ± S.E.M
LF	WT	2.1 ± 0.05	0.69 ± 0.19
LF	Apo E KO	*6.95 ± 1.97	0.68 ± 0.09
HF	WT	2.2 ± 0.46	0.42 ± 0.12
HF	Apo E KO	*14.3 ± 0.01	0.38 ± 0.12

*$P < 0.05$
S.E.M = standard error of the mean
Table shows plasma cholesterol and triglyceride concentrations (mean ± SEM, n = 6 mice per group) in C57BL/6J WT mice and apo E KO mice maintained on either LF or HF diet for six-months. Apo E KO mice had significantly elevated levels of plasma cholesterol compared to WT controls under both feeding regimens ($P < 0.05$). High-fat feeding further exacerbated the elevation of cholesterol in apo E KO mice compared to HF-WT ($P < 0.001$) and LF-APOE KO mice ($P < 0.05$). Plasma triglyceride was not significantly different between groups.

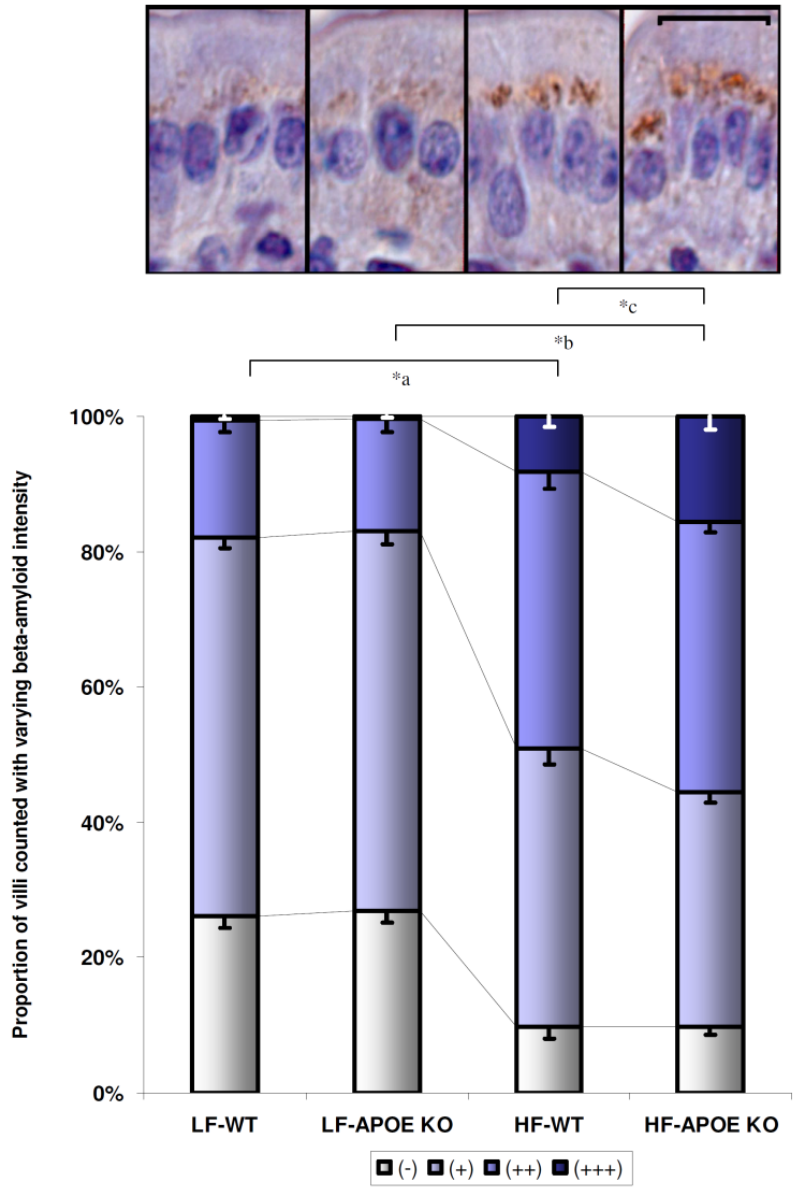


Figure 1

Enterocytic Aβ in wild-type and apolipoprotein E knockout mice given a high fat diet. Figure shows proportion of small intestinal epithelial cells with different staining intensity for Aβ. Score as follows: (-) no granular coloration, (+) modest with 1–2 granules, (2+) moderate with 3–4 granules or (3+) high, containing larger intense granules. Data was collected for six mice per group, with a minimum of four tissue sections per mouse studied. A minimum of 200 cells per section were scored and statistical significance was determined by one-way ANOVA with post-hoc Bonferroni test. LF-WT and LF-APOE KO mice have significantly ($p < 0.05$) fewer cells which stained positive for Aβ compared to mice fed high fats (HF-WT and HF-APOE KO *a and *b respectively). Under high-fat feeding, apo E KO mice had significantly greater proportion of cells which expressed Aβ at higher intensity compared to high-fat fed WT mice (*c, $p < 0.05$). The inset micrograph shows high-magnification of enterocytes from groups corresponding to graphs below. Beta-amyloid colocalized within the perinuclear regions of the cell containing Golgi and ER within enterocytes from all groups. (Scale bar = 20 μm).

exhibited positive staining of Aβ relatively evenly distributed throughout the mucosa epithelium. Apo E KO mice on low-fat diets showed a similar distribution of Aβ compared to WT controls (figure 1). The effect of high-fat feeding on enterocytic Aβ in WT and apo E KO mice is also given in figure 1. Both WT and apo E KO mice had significantly greater enterocytic Aβ abundance, however the effect was more pronounced in the apo E knockout group, notably with more enterocytes showing intense (3+) staining (double asterisks, figure 1).

Villi height in apo E KO mice and effects of high fat feeding

Small intestinal villi length was determined as a surrogate marker of intestinal absorptive capacity. Apo E KO mice on the low-fat diet had significantly greater mean villi length compared to WT controls (figure 2). High-fat feeding was found to substantially increase villi length in control animals and was comparable to apo E KO mice. High-fat feeding had no synergistic influence on villi length in the absence of apo E expression (figure 2).

Discussion

This study shows that in the absence of apo E, intestinal villi length is significantly greater than WT mice. The absence of apo E coupled with chronic ingestion of a saturated fat and cholesterol diet, increased enterocytic Aβ abundance compared to WT mice on a low-fat diet. This may have simply been a dietary-fat induced effect independent of apo E, because apo E KO mice on a low-fat diet showed similar levels of enterocytic Aβ compared to WT controls. On the other hand, the absence of apo E with a high-fat diet was found to enhance Aβ abundance above that observed in WT mice given saturates and cholesterol. The latter is consistent with modulation of Aβ by apo E that is lipid-threshold dependent.

Apolipoprotein E serves as a TRL ligand for both the LDL receptor and LRP [24,25]. The liver is a major source of apo E, however other tissues including the small intestine express apo E [24,26,27]. Apo E KO mice [28-30] accumulate TRL's because they are unable to bind and be cleared by receptor processes [31]. Under low-fat feeding, apo E KO mice had a greater than three-fold increase in plasma. High-fat feeding exacerbated plasma cholesterol accumulation in apo E KO mice, presumably because of exaggerated lipoprotein production and indeed hypercholesterolemia was increased two-fold above low-fat fed apo E KO mice. Clearance of TRL's from blood is a two-step process requiring triglyceride lipolysis by lipases to produce a depleted apo E rich 'remnant' lipoprotein [24]. Thereafter, remnants are cleared by receptor pathways utilizing apo E as the ligand. There is no hydrolytic defect in apo E KO mice, which explains why these mice were not hypertriglyceridemic.

The mechanisms by which the absence of apo E increased enterocytic Aβ in high-fat fed mice are unclear, although studies in cell culture provide clues. Irizarry *et al* (2004) found that incubation of neuronal cells with apo E resulted in a reduced synthesis of Aβ by lowering the gamma secretase activity [4]. Rough endoplasmic reticulum (rER) and the Golgi compartments are where early endoplasmic cleavage of the Aβ precursor protein occurs, the latter consistent with increased enterocytic perinuclear Aβ immunostaining in apo E KO mice.

This study and others [32,33] found longer villi length in apo E KO mice, suggestive of greater absorptive capacity. Greater substrate availability might stimulate Aβ biogenesis and this hypothesis is supported by the increase in Aβ abundance in high-fat WT mice which also had a marked increase in villus length. Greater Aβ abundance would have been expected in apo E KO mice given the low-fat diet compared to WT controls, because villus length was comparatively greater in the absence of the apo E gene. However, if lipid absorption is already efficient with the low-fat feeding regimen; the deletion of apo E (and increased villus length) would not necessarily have had the expected stimulatory effect on enterocytic Aβ.

Chylomicron synthesis occurs within the ER and Golgi requiring the progressive lipidation of apolipoprotein B_{48} (apo B_{48}) [34,35]. Dietary fats transiently stimulate chylomicron synthesis and secretion [36,37] and in clinical studies post-prandial elevations in the Aβ-precursor protein have been reported synergistic with the lipaemic response [3]. How Aβ binds and is secreted with chylomicron is unclear, although the protein is known to bind avidly with negatively charged hydrophobic lipids [5,38]. Cell culture studies also support a lipoprotein mediated secretory pathway because in hepatocyte media, Aβ is found associated with lipoprotein complexes [11].

In animal models and in cell cultures, apo E has confounding effects on hepatic secretion of VLDL. Apo E will normally suppress apo B production, but this is contradicted in the presence of lipids which strongly stimulate lipoprotein biogenesis [39]. In this study, enterocytic Aβ abundance was not significantly different in low-fat apo E KO mice compared to controls, suggesting that chylomicron synthetic rates were not different between these two groups of mice. The increased availability of dietary lipids when animals were fed the high-fat diet would promote chylomicron production and by extension, perhaps Aβ genesis. However, whilst enhanced enterocytic abundance of Aβ was seen in both WT and apo E KO mice given the high-fat diet, the effect was greater in the latter. One explanation is the finding that apo E normally suppresses triglyceride secretion from liver. Therefore, the enhanced effect on enterocytic Aβ seen in apo E KO given high-fat

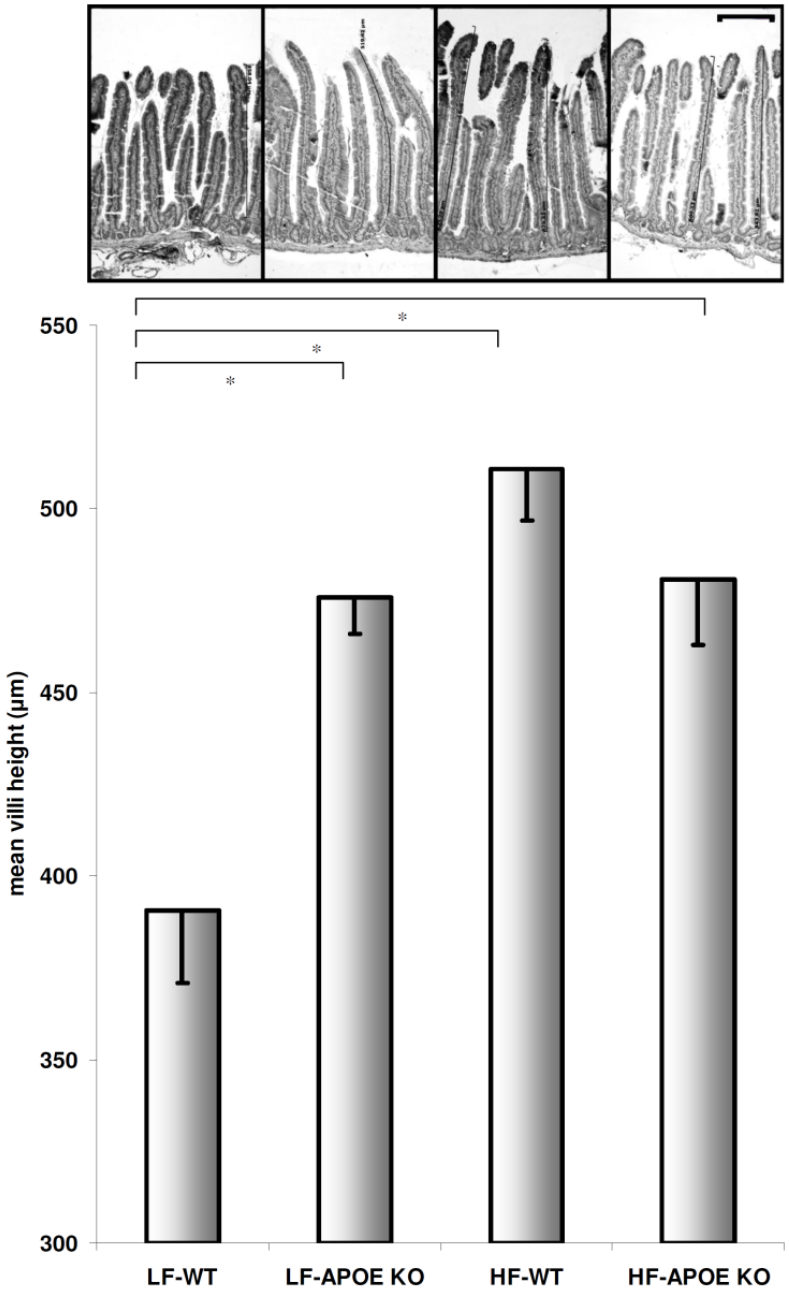


Figure 2
Villi height in wild-type and apolipoprotein E knockout mice given a high fat diet. Mean villi height (mm) in WT and apo E KO mice fed low- and high-fat chow. LF-WT group had significantly (*$p < 0.05$) shorter villi height compared to other groups. The inset micrograph shows low-magnification of intestinal villi height for each group. (Scale bar = 200 µm).

may have been indicative of amplification in the presence of greater cytosolic lipids [39,40].

Conclusion

Many studies have demonstrated the central role of apo E in maintaining cerebral Aβ homeostasis including modulation of production, as a chaperone protein, and in maintaining efflux and influx pathways across the blood brain barrier. Furthermore, apo E profoundly influences the kinetics in blood of Aβ containing lipoproteins as well as their secretion from liver. This study now demonstrates that apo E may also regulate intestinal Aβ metabolism.

Materials and methods

Animals

The protocols described were approved by an ethics committee accredited by the National Health and Medical Research Council of Australia (Curtin University ethics approval N 55-04). Six-week-old female C57BL/6J apolipoprotein E gene knockout (apo E KO) and wild-type (WT) mice weighing approximately 16 g were obtained from the Animal ARC, Perth, Western Australia. Mice were divided and randomly allocated into a low-fat or high-fat diet group. Mice were housed separately in a well-ventilated room that was maintained at 22°C on a 12:12-h light/dark cycles. Body weight was measured weekly.

Dietary regimen

Chow was purchased from Rodent Diet Specialty Feeds (Glen Forrest, Western Australia). The low-fat (control) group of mice was given chow that contained 4.0% (w/w) as unsaturated fat (AIN93M standard rodent diet) and the diet was free of cholesterol. Mice on the high-fat diet were given chow containing 1.0% (w/w) as unsaturated fat and 16.0% (w/w) as saturated fat (SF00–245 high-fat mouse diet). In addition, the high-fat feed was supplemented with 1% (w/w) cholesterol and 0.5% (w/w) cholate, the latter to aid in absorption. The digestible energy for low-fat and high-fat feed were 15.2 MJ/kg and 18.7 MJ/kg respectively. Food and water were available *ad libitum*.

Sample collection

After six-months of dietary interventions, mice were anaesthetized with an intraperitoneal injection of Phenobarbital (45 mg/kg). Mice were exsanguinated by cardiac punctureand blood was collected into ethylene-diaminetetracetic acid (EDTA)-tubes. Plasma was separated by low speed centrifugation and stored at -80°C (under an atmosphere of argon).

Tissue processing

A small intestine segment measuring 2 cm was cut and isolated from the rest of the digestive tract at the proximal duodenal sphincter. The contents were flushed *in-situ* with phosphate buffered saline (PBS, pH = 7.4), and placed into 10% buffered formalin (ph = 7.4) for fixation. Tissues were fixed for 24 h and processed for immunohistochemistry (IHC).

Immunohistochemistry

Tissue sections (5 μm) were deparaffinised, rehydrated and IHC analysis was done as previously described [21]. Briefly, the sections were exposed to 3% hydrogen peroxide in methanol for 30 min to quench endogenous peroxidase activity, washed and incubated in blocking serum (20% goat serum) prior to overnight incubation at 4°C with polyclonal rabbit anti-human $A\beta_{1-40/42}$ antiserum (AB5076, Chemicon Temecula, CA), diluted to 1:1000 with 10% goat serum. We previously established specificity by replacing the primary antibody with an irrelevant serum or with PBS and by competition IHC analysis [21]. For the latter, the primary antisera were pre-mixed with solubilised Aβ. Cerebral tissues from transgenic mice (Tg2576sw) expressing familial human APP695 with established plaques were used as positive controls. Slides were washed in PBS and incubated with biotinylated goat anti-rabbit secondary antibody (1:1000 dilution) (E 0432, DAKO, Carpinteria, CA), followed by avidin-biotin-peroxidase complex (ABC/HRP) (K 0377, DAKO, Carpinteria, CA) for 45 min at room temperature. Positive immunostaining was established with liquid diaminobenzidine plus (DAB+) substrate chromogen kit (K 3467, DAKO, Carpinteria, CA). Sections were then counterstained with Harris's haematoxylin.

Imaging

Digital images for photomicroscopy were acquired by AxioCam HRc camera (Zeiss Germany). Images were captured under identical settings utilising AxioVision software, version 4.5.

Quantitation of intestinal beta-amyloid abundance

Six animals per group were investigated with a minimum of four tissue blocks prepared for each. From each slide, four images were captured randomly at low magnification (Zeiss AxioVert 200 M, Germany). The intensity of immunolabeling was quantified as previously described [41,42]. Labelling was considered adequate if it was mild (+), moderate (2+), or intense (3+), with adequately labeled positive controls and no labeling in negative controls. The total number of cells with different intensity of Aβ staining was counted by a blinded-to-group investigator in each villus and the data expressed as a percentage.

Measurements of intestinal villi height

Total of 8 images was taken at low-magnification per group. Representative villi were selected by two independent investigators for height measurement (measurement tool, AxioVision program 4.5).

Plasma lipid measurements

Plasma lipids were measured immediately following plasma isolation via commercial absorbance-based assays. Triglyceride was determined by measurement of glycerol liberated following enzymatic hydrolysis of triglyceride (TR 1697, Randox laboratories, U.K). Total plasma cholesterol concentration was determined via the cholesterol esterase/cholesterol oxidase technique (CH 201, Randox laboratories, U.K).

Statistical analysis

The effect of high-fat feeding and apo E gene on Aβ abundance, intestinal villi height, plasma triglyceride and total cholesterol was assessed by univariate analysis. Post-hoc comparisons of means were performed using Bonferroni tests and if equal variance was not found, then Games-Howell test was used to compare difference between individual groups. P-value < 0.05 was considered a statistically significant.

List of abbreviations

Aβ: beta-amyloid; AD: Alzheimer's disease; Apo: apolipoprotein; APP: amyloid precursor protein; CH: cholesterol; IHC: immunohistochemistry; KO: knockout; TG: triglycerides; TRL: triglyceride-rich-lipoprotein; WT: wild-type.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

SG participated in the design of the study, carried out the study, performed the IHC and lipid analysis and helped to draft the manuscript. MMSPG helped to collect data for results analysis. RT helped to collect data for results analysis. LJ participated in the design of study and performed the statistical analysis. RDJ participated in design of study and helped to draft the manuscript. SSD helped with performing statistical analysis. JCLM participated in the design of the study, performed statistical analysis, and coordinated and helped to draft the manuscript. All authors read and approved the final manuscript.

Acknowledgements

The authors would like to thank Columbur Cheung and Karl Fairhurst for technical assistance.

References

1. DeMattos RB: **Apolipoprotein E dose-dependent modulation of beta-amyloid deposition in a transgenic mouse model of Alzheimer's disease.** *J Mol Neurosci* 2004, **23:**255-262.
2. Bales KR, Verina T, Cummins DJ, Du Y, Dodel RC, Saura J, Fishman CE, DeLong CA, Piccardo P, Petegnief V, Ghetti B, Paul SM: **Apolipoprotein E is essential for amyloid deposition in the APP(V717F) transgenic mouse model of Alzheimer's disease.** *Proc Natl Acad Sci U S A* 1999, **96:**15233-15238.
3. Boyt AA, Taddei K, Hallmayer J, Mamo J, Helmerhorst E, Gandy SE, Martins RN: **Relationship between lipid metabolism and amyloid precursor protein and apolipoprotein E.** *Alzheimer's Reports* 1999, **2:**339-346.
4. Irizarry MC, Deng A, Lleo A, Berezovska O, Von Arnim CA, Martin-Rehrmann M, Manelli A, LaDu MJ, Hyman BT, Rebeck GW: **Apolipoprotein E modulates gamma-secretase cleavage of the amyloid precursor protein.** *J Neurochem* 2004, **90:**1132-1143.
5. James AP, Pal S, Gennat HC, Vine DF, Mamo JC: **The incorporation and metabolism of amyloid-beta into chylomicron-like lipid emulsions.** *J Alzheimers Dis* 2003, **5:**179-188.
6. LaDu MJ, Lukens JR, Reardon CA, Getz GS: **Association of human, rat, and rabbit apolipoprotein E with beta-amyloid.** *J Neurosci Res* 1997, **49:**9-18.
7. Strittmatter WJ, Weisgraber KH, Huang DY, Dong LM, Salvesen GS, Pericak-Vance M, Schmechel D, Saunders AM, Goldgaber D, Roses AD: **Binding of human apolipoprotein E to synthetic amyloid beta peptide: isoform-specific effects and implications for late-onset Alzheimer disease.** *Proc Natl Acad Sci U S A* 1993, **90:**8098-8102.
8. Ye S, Huang Y, Mullendorff K, Dong L, Giedt G, Meng EC, Cohen FE, Kuntz ID, Weisgraber KH, Mahley RW: **Apolipoprotein (apo) E4 enhances amyloid beta peptide production in cultured neuronal cells: apoE structure as a potential therapeutic target.** *Proc Natl Acad Sci U S A* 2005, **102:**18700-18705.
9. Deane R, Du Yan S, Submamaryan RK, LaRue B, Jovanovic S, Hogg E, Welch D, Manness L, Lin C, Yu J, Zhu H, Ghiso J, Frangione B, Stern A, Schmidt AM, Armstrong DL, Arnold B, Liliensiek B, Nawroth P, Hofman F, Kindy M, Stern D, Zlokovic B: **RAGE mediates amyloid-beta peptide transport across the blood-brain barrier and accumulation in brain.** *Nat Med* 2003, **9:**907-913.
10. Dodart JC, Bales KR, Gannon KS, Greene SJ, DeMattos RB, Mathis C, DeLong CA, Wu S, Wu X, Holtzman DM, Paul SM: **Immunization reverses memory deficits without reducing brain Abeta burden in Alzheimer's disease model.** *Nat Neurosci* 2002, **5:**452-457.
11. Koudinov AR, Koudinova NV: **Alzheimer's soluble amyloid beta protein is secreted by HepG2 cells as an apolipoprotein.** *Cell Biol Int* 1997, **21:**265-271.
12. Mahley RW: **Apolipoprotein E: cholesterol transport protein with expanding role in cell biology.** *Science* 1988, **240:**622-630.
13. . .
14. Morris MC, Evans DA, Bienias JL, Tangney CC, Bennett DA, Aggarwal N, Schneider J, Wilson RS: **Dietary fats and the risk of incident Alzheimer disease.** *Arch Neurol* 2003, **60:**194-200.
15. Kalmijn S, Launer LJ, Ott A, Witteman JC, Hofman A, Breteler MM: **Dietary fat intake and the risk of incident dementia in the Rotterdam Study.** *Ann Neurol* 1997, **42:**776-782.
16. Engelhart MJ, Geerlings MI, Ruitenberg A, Van Swieten JC, Hofman A, Witteman JC, Breteler MM: **Diet and risk of dementia: Does fat matter?: The Rotterdam Study.** *Neurology* 2002, **59:**1915-1921.
17. Luchsinger JA, Mayeux R: **Dietary factors and Alzheimer's disease.** *Lancet Neurol* 2004, **3:**579-587.
18. Solfrizzi V, D'Introno A, Colacicco AM, Capurso C, Del Parigi A, Capurso S, Gadaleta A, Capurso A, Panza F: **Dietary fatty acids intake: possible role in cognitive decline and dementia.** *Exp Gerontol* 2005, **40:**257-270.
19. Sparks DL, Scheff SW, Hunsaker JC 3rd, Liu H, Landers T, Gross DR: **Induction of Alzheimer-like beta-amyloid immunoreactivity in the brains of rabbits with dietary cholesterol.** *Exp Neurol* 1994, **126:**88-94.
20. Refolo LM, Malester B, LaFrancois J, Bryant-Thomas T, Wang R, Tint GS, Sambamurti K, Duff K, Pappolla MA: **Hypercholesterolemia accelerates the Alzheimer's amyloid pathology in a transgenic mouse model.** *Neurobiol Dis* 2000, **7:**321-331.
21. Shie FS, Jin LW, Cook DG, Leverenz JB, LeBoeuf RC: **Diet-induced hypercholesterolemia enhances brain A beta accumulation in transgenic mice.** *Neuroreport* 2002, **13:**455-459.
22. Galloway S, Le J, Johnsen R, Chew S, Mamo JCL: **Beta-amyloid or its precursor protein is found in epithelial cells of the small intestine and stimulated by high fat feeding.** *Journal of Nutritional Biochemistry* 2006, **In press:**.
23. Su GC, Arendash GW, Kalaria RN, Bjugstad KB, Mullan M: **Intravascular infusions of soluble beta-amyloid compromise the blood-brain barrier, activate CNS glial cells and induce peripheral hemorrhage.** *Brain Res* 1999, **818:**105-117.
24. Mahley RW, Hui DY, Innerarity TL, Weisgraber KH: **Two independent lipoprotein receptors on hepatic membranes of dog, swine, and man. Apo-B,E and apo-E receptors.** *J Clin Invest* 1981, **68:**1197-1206.

25. Hui DY, Innerarity TL, Mahley RW: **Lipoprotein binding to canine hepatic membranes. Metabolically distinct apo-E and apo-B,E receptors.** *J Biol Chem* 1981, **256:**5646-5655.
26. Driscoll DM, Getz GS: **Extrahepatic synthesis of apolipoprotein E.** *J Lipid Res* 1984, **25:**1368-1379.
27. Emanuele E, Peros E, Tomaino C, Feudatari E, Bernardi L, Binetti G, Maletta R, D'Angelo A, Montagna L, Bruni AC, Geroldi D: **Apolipoprotein(a) null phenotype is related to a delayed age at onset of Alzheimer's disease.** *Neurosci Lett* 2004, **357:**45-48.
28. Zhang SH, Reddick RL, Piedrahita JA, Maeda N: **Spontaneous hypercholesterolemia and arterial lesions in mice lacking apolipoprotein E.** *Science* 1992, **258:**468-471.
29. Plump AS, Smith JD, Hayek T, Aalto-Setala K, Walsh A, Verstuyft JG, Rubin EM, Breslow JL: **Severe hypercholesterolemia and atherosclerosis in apolipoprotein E-deficient mice created by homologous recombination in ES cells.** *Cell* 1992, **71:**343-353.
30. Nakashima Y, Plump AS, Raines EW, Breslow JL, Ross R: **ApoE-deficient mice develop lesions of all phases of atherosclerosis throughout the arterial tree.** *Arterioscler Thromb* 1994, **14:**133-140.
31. Hultin M, Olivecrona T: **Conversion of chylomicrons into remnants.** *Atherosclerosis* 1998, **141(Suppl 1):**25-29.
32. Kanemitsu N, Shimamoto C, Hiraike Y, Omae T, Iwakura K, Nakanishi Y, Katsu K: **Fat Absorption and Morphological Changes in the Small Intestine in Model Mice with Hyperlipidemia (Apo E Deficiency).** *Bulletin of the Osaka Medical College* 2006, **52:**59-67.
33. Kesaniemi YA, Ehnholm C, Miettinen TA: **Intestinal cholesterol absorption efficiency in man is related to apoprotein E phenotype.** *J Clin Invest* 1987, **80:**578-581.
34. Hussain MM, Kedees MH, Singh K, Athar H, Jamali NZ: **Signposts in the assembly of chylomicrons.** *Front Biosci* 2001, **6:**D320-31.
35. van Greevenbroek MM, van Meer G, Erkelens DW, de Bruin TW: **Effects of saturated, mono-, and polyunsaturated fatty acids on the secretion of apo B containing lipoproteins by Caco-2 cells.** *Atherosclerosis* 1996, **121:**139-150.
36. van Greevenbroek MM, de Bruin TW: **Chylomicron synthesis by intestinal cells in vitro and in vivo.** *Atherosclerosis* 1998, **141 Suppl 1:**S9-16.
37. Luchoomun J, Hussain MM: **Assembly and secretion of chylomicrons by differentiated Caco-2 cells. Nascent triglycerides and preformed phospholipids are preferentially used for lipoprotein assembly.** *J Biol Chem* 1999, **274:**19565-19572.
38. Shao H, Jao SC, Ma K, Zagoriski MG: **Solution Structures of Michelle-bound Amyloid beta1-40 and bet1-42 Peptides of Alzheimer's Disease.** *J Mol Biol* 1999, **285:**755-773.
39. Maugeais C, Tietge UJ, Tsukamoto K, Glick JM, Rader DJ: **Hepatic apolipoprotein E expression promotes very low density lipoprotein-apolipoprotein B production in vivo in mice.** *J Lipid Res* 2000, **41:**1673-1679.
40. Mensenkamp AR, Van Luyn MJ, Havinga R, Teusink B, Waterman IJ, Mann CJ, Elzinga BM, Verkade HJ, Zammit VA, Havekes LM, Shoulders CC, Kuipers F: **The transport of triglycerides through the secretory pathway of hepatocytes is impaired in apolipoprotein E deficient mice.** *J Hepatol* 2004, **40:**599-606.
41. Mikaelian I, Nanney LB, Parman KS, Kusewitt DF, Ward JM, Naf D, Krupke DM, Eppig JT, Bult CJ, Seymour R, Ichiki T, Sundberg JP: **Antibodies that label paraffin-embedded mouse tissues: a collaborative endeavor.** *Toxicol Pathol* 2004, **32:**181-191.
42. Donahue JE, Flaherty SL, Johanson CE, Duncan JA 3rd, Silverberg GD, Miller MC, Tavares R, Yang W, Wu Q, Sabo E, Hovanesian V, Stopa EG: **RAGE, LRP-1, and amyloid-beta protein in Alzheimer's disease.** *Acta Neuropathol* 2006, **112:**405-415.

CHAPTER 4

Chapter 4 – Colocalization of intestinal Aβ with apo B

The content of this chapter will be covered by article 3:

Galloway S, Takechi R, Pallebage-Gamarallage MM, Dhaliwal SS, Mamo JC. (2009) Amyloid-beta colocalizes with apolipoprotein B in absorptive cells of the small intestine. *Lipids Health Dis.* 8, 46.

Objective addressed:

Objective 4: To ascertain whether intestinal Aβ associates with apo B within the enterocytes of the small intestines. Secondly, to further determine if high fat feeding can modulate the degree of association between intestinal Aβ and apo B.

Hypothesis: High fat feeding will increase the association between Aβ and apolipoprotein B in enterocytes.

Article summary:

It is clear from previous studies that intestinal expression of Aβ is regulated by dietary fat intake. We have previously shown high fat feeding increased Aβ abundance compared to low-fat groups whilst fasting abolished expression. Immunolocalisation of Aβ expression within enterocytes occurs specifically above the nucleus in “perinuclear” location, at the site of proteins and CM synthesis. Several lines of evidence suggest that Aβ can bind to and behave as a regulatory apolipoprotein component of CMs. There exists a possibility that Aβ can form a part of primordial lipoprotein during the process of lipidation, however it is not currently known if this happens and if so, to what extent. This study utilizes 3D double IF to determine if enterocytic Aβ associates with intestinal lipoprotein CMs.

A significant abundance of apo B (an obligatory component of CMs) and Aβ was found within the perinuclear location of enterocytes. Both apo B and Aβ co-localised with the Golgi apparatus. Patterns of Aβ and apo B remained essentially the same in high and low-fat feeding groups but the abundance was almost double

for high fat groups. Co-localization studies show that 73 % of Aβ associates with apo B increasing to 87 % under high fat feeding. This study provides in vivo evidence that Aβ is secreted as a CM complex within enterocytes and explores the plasma kinetics of plasma Aβ-lipoprotein in context with the association between high fat diet and AD risk.

Lipids in Health and Disease

BioMed Central

Research

Open Access

Amyloid-β colocalizes with apolipoprotein B in absorptive cells of the small intestine

Susan Galloway, Ryusuke Takechi, Menuka MS Pallebage-Gamarallage, Satvinder S Dhaliwal and John CL Mamo*

Address: The Australian Technology Network Centre for Metabolic Fitness, School of Public Health, Curtin Health Innovation Research Institute, Curtin University of Technology, Perth, Western Australia, Australia

Email: Susan Galloway - susan.galloway@postgrad.curtin.edu.au; Ryusuke Takechi - ryusuke.takechi@postgrad.curtin.edu.au; Menuka MS Pallebage-Gamarallage - m.pallebag@postgrad.curtin.edu.au; Satvinder S Dhaliwal - S.Dhaliwal@Curtin.edu.au; John CL Mamo* - J.Mamo@Curtin.edu.au

* Corresponding author

Published: 22 October 2009

Lipids in Health and Disease 2009, **8**:46 doi:10.1186/1476-511X-8-46

Received: 10 September 2009
Accepted: 22 October 2009

This article is available from: http://www.lipidworld.com/content/8/1/46

Abstract

Background: Amyloid-β is recognized as the major constituent of senile plaque found in subjects with Alzheimer's disease. However, there is increasing evidence that in a physiological context amyloid-β may serve as regulating apolipoprotein, primarily of the triglyceride enriched lipoproteins. To consider this hypothesis further, this study utilized an in vivo immunological approach to explore in lipogenic tissue whether amyloid-β colocalizes with nascent triglyceride-rich lipoproteins.

Results: In murine absorptive epithelial cells of the small intestine, amyloid-β had remarkable colocalization with chylomicrons (Manders overlap coefficient = 0.73 ± 0.03 (SEM)), the latter identified as immunoreactive apolipoprotein B. A diet enriched in saturated fats doubled the abundance of both amyloid-β and apo B and increased the overlap coefficient of the two proteins (0.87 ± 0.02). However, there was no evidence that abundance of the two proteins was interdependent within the enterocytes (Pearson's Coefficient < 0.02 ± 0.03), or in plasma (Pearson's Coefficient < 0.01).

Conclusion: The findings of this study are consistent with the possibility that amyloid-β is secreted by enterocytes as an apolipoprotein component of chylomicrons. However, secretion of amyloid-β appears to be independent of chylomicron biogenesis.

Background

Amyloid-β is recognized as the principal protein in senile plaques in subjects with Alzheimer's disease (AD) [1]. Generated from the slicing of amyloid precursor protein (βAPP) by secretases, the synthesis of amyloid-β can be differentially modulated by cellular lipid homeostasis. Studies in cell culture and in vivo suggest that cholesterol inhibits amyloid-β biogenesis [2-4], although this effect may be dependent on the distribution of free and esterified cholesterol within the plasma membrane and within lipid rafts [5]. In contrast, in vivo studies found that chronic ingestion of diets enriched in saturated-fats (SFA) had a potent stimulatory effect on enterocytic amyloid-β abundance [6].

Several lines of evidence suggest that one physiological role for amyloid-β is as a regulating apolipoprotein, particularly of the triglyceride-rich lipoproteins (TRL's). Koudinov et al reported that amyloid-β is secreted by hepatocytes as a lipoprotein complex [7]. Significant plasma abundance of amyloid-β was also found in the TRL fraction of control subjects and amyloid-β enrichment in TRL's was evident in subjects with AD, or with mild cognitive impairment [8]. Ingestion of a lipid rich meal also causes a transient increase in plasma of soluble APP, concomitant with postprandial lipaemia [9] and when injected intravenously associated with TRL-emulsions, amyloid-β increased uptake in fat-rich tissues relative to liver [10].

The βAPP is expressed on the plasma membrane of a number of tissues including lipogenic organs such as liver [6]. Proteolytic cleavage of βAPP generally results in the extracellular release of amyloid-β which is then chaperoned by transporter proteins [11-14]. However, hydrophobic domains within amyloid-β [15] results in rapid folding of amyloid-β that make it unlikely to readily associate with lipoproteins already secreted into circulation. Rather, immunhistochemistical studies show amyloid-β abundance within the perinuclear region of hepatocytes and absorptive epithelial cells of the small intestine [4,6,7,16], suggesting that amyloid-β may form part of the primordial lipoprotein during the lipidation process. Consistent with the latter, using a phage display Nelson and Alkon showed that amyloid-β bound tightly with several apolipoproteins found commonly with TRL [17]. To further consider the possibility that amyloid-β becomes associated with nascent lipoproteins, in this study we utilized sensitive three-dimensional (3D) immunofluorescent (IF) microscopy to explore if enterocytic abundance of amyloid-β is associated with chylomicrons. Apolipoprotein (apo) B, an obligatory component of TRL secreted by intestine and liver, was used as a marker of enterocytic chylomicron distribution and plasma abundance of TRL.

Materials and methods

Diet and animals

The protocols described in this study were approved by an accredited National Health and Medical Research Council of Australia Animal Ethics Committee (Curtin University Animal Experimentation and Ethics Committee Reference number R02-07). Six-week-old female C57BL/6J mice (Animal resources centre, Murdoch, Western Australia) were divided randomly into a low-fat (LF) or saturated fatty acid (SFA) diet group. Low fat mice were given chow that contained 3.6% (w/w) as unsaturated fat and 0.4% SFA (AIN93M, Specialty Feeds, Western Australia). The SFA enriched chow contained 12.9% (w/w) as saturated fats and 7.4% as unsaturated oils (SF07-50, Specialty feeds, Western Australia). Both diets were free of cholesterol. Digestible energy for LF and SFA feed were 15.1 MJ/kg and 18.8 MJ/kg respectively and feed was available *ad libitum*. After three-months of dietary intervention, mice were sacrificed by pentobarbital injection. The small intestine was isolated and flushed with chilled phosphate buffered saline (PBS, pH 7.4). A 2 cm segment of the small intestine distal to the duodenum was fixed in 4% paraformaldehyde for a minimum of 24 h, processed and longitudinal segments embedded in paraffin wax. Serial sections of 5 μm thick were cut on microtome and mounted on silanised slides for histology and immunofluorescence microscopy.

Antibodies

Anti-apo B, anti Golgi-apparatus (anti-Golgi 58 K), anti-rabbit IgG with Alexa488, and streptavidin-Alexa546 were obtained from Invitrogen (Melbourne, Victoria, Australia). Anti-rabbit IgG biotin conjugate was obtained from DAKO (Glostrup, Denmark). Rabbit anti-human amyloid-β was obtained from Chemicon International (Temecula, California, United States).

Double-immunofluorescent labelling

An established double IF labelling method was utilized as previously described [18]. Cross reactivity was prevented using a biotin-avidin amplification technique microscopy. The concentration of the primary antibody used with biotin-avidin amplification is substantially below the threshold required for detection by standard IF and does not interfere with detection of the second protein.

Anti-amyloid-β (1:1000) was added to sections overnight at 4°C, followed by addition of goat anti-rabbit IgG with biotin (1:200) for 1 h at room temperature. Thereafter, anti-Golgi-apparatus (1:10) was added overnight at 4°C. Immunofluorescence was detected by streptavidin-Alexa546 (1:100) and anti-rabbit IgG with Alexa488 (1:100) for amyloid-β and Golgi-apparatus respectively. Cell nuclei were detected using DAPI and slides were mounted using anti-fade mounting medium. The same method was used to achieve double apo B and Golgi-apparatus staining by substituting the anti-amyloid-β with anti-apo B (1:400).

Image capture

Digital images were captured using AxioCam mRM and ApoTome on a Zeiss Axiovert 200 M inverted microscope and visualized with Plan-NeoFluar lenses (Carl Zeiss, Oberkochen, Germany). Excitation and emission were achieved by using filters 43 (Ex BP545/25, beam splitter FT570 and Em BP605/70) and 38 (Ex BP470/40, beam splitter FT495 and Em BP525/50) to determine fluorescence of Alexa546 and Alexa488 respectively. Filter 49 (Ex G365, beam splitter FT 395 and Em BP445/50) was used to detect nuclei stain DAPI. Individual channels are

devoid of fluorescence from other emission sources and are therefore clear of bleed-through.

Three-dimensional images were captured using the ApoTome optical sectioning mode which allows the creation of a 3D image based on the 'stacking' of consecutive 2D images. Each 3D image consisted from 8-10 2D images, and the axial distance of Z-stack was 0.5 μm for 200×. There were 6 animals per group with a minimum of 40 images per mouse used for analysis. Fluorescent intensity and area were determined using the measurement and colocalization module available on AxioVision v4.7.1 software (Carl Zeiss, Oberkochen, Germany).

Quantification of fluorescent intensity and colocalization

There are several algorithms capable of achieving measures of colocalization or association via measurement of fluorescent pixel spatial orientation and pixel intensity. The Pearson's correlation coefficient (r) is a commonly used quantitative estimate of association (abundance) for proteins [19]. However, as Pearson's correlation is a measure of variance from the mean pixel intensity, it does not provide information of the area of overlap. A modification to Pearson's correlation coefficient developed by Manders et al (1993) eliminates the average grey values from the Pearson's formula to allow the quantification of overlapping pixels from each channel [19]. The degree of colocalization for the proteins is positively related to the Manders coefficient, known commonly as the 'overlap coefficient' (OC). The AxioVision software utilizes an automated procedure based on spatial statistics to determine Pearson's correlation coefficient and Manders OC, thereby avoiding selection bias by manual selection methods.

Western blotting for plasma apolipoprotein B

Plasma samples were separated on NuPAGE 3-8% Tris-acetate gels (EA03752BOX, Invitrogen, Victoria, Australia) at 150 V (Biorad Model 20012.0) for 1 hr. Gels were then electrotransferred to PVDF membranes (PV4HY00010, Osmonics Inc, Minnesota U.S.A) at 40 V for 1 hr and blocked in 10% skim milk (in TBST) overnight at 4°C. The membranes were incubated with polyclonal rabbit anti-human apo B 1:100 (Q0497, Dakocytomation, Glostrup, Denmark), and then with donkey anti-rabbit immunoglobulin G (IgG) horseradish peroxidase (HRP) (Na934V, Amersham Bioscience, Buckinghamshire, UK). Proteins were detected using enhanced chemiluminescence reagent (ECL™) western blotting analysis system (RPN2108, Amersham Bioscience, Buckinghamshire, UK). Membranes were exposed to high performance chemiluminescence film (Amersham Hyperfilm™, Amersham Bioscience, Buckinghamshire, UK) and developed in an AGFA-Gevaert Rapidoprint X-Ray Developer (Septestraat, Belgium). Apo B48 bands were identified and quantified by densitometry against purified apo B48 protein of known mass (550 kDa for apo B-100 and 260 kDa for apo B48).

Amyloid-beta ELISA

Plasma amyloid-β 40/42 levels were measured using commercially available ELISA kits (Biosource, Camarillo CA).

Statistics

Enterocytic colocation of amyloid-β with apo B was determined by an automated procedure based on spatial statistics to determine Pearson's correlation coefficient and Manders OC (AxioVision 4.0). The association between total apo B, apo B48, and apo B100 with total amyloid-β, amyloid-β40 and amyloid-β42 were examined using Pearson's and Spearman's correlation. Spearman's correlation was used when the assumptions of the analysis were violated due to the presence of outliers. P-values less than 5% were considered as statistically significant and the data was analysed using SPSS version 17.0.

Results

Enterocytic chylomicrons were detected by determining the distribution of apo B, an obligatory structural component of chylomicrons. Significant amounts of amyloid-β and apo B were found to be enriched within the perinuclear region of cells. Amyloid-β and apo B colocalized with the Golgi-apparatus, towards the basolateral surface of the cell and within the lacteals (Figure 1). The patterns of distribution for amyloid-β and apo B remained essentially the same in LF and SFA fed mice (Figure 2), however abundance of each protein more than doubled in SFA fed mice compared to LF fed animals (Table 1, columns 1 and 2).

The colocalization of enterocytic amyloid-β and apo B was expressed as the OC (Manders overlap coefficient). The relative abundance of amyloid-β and apo B in LF and in SFA fed mice, given as mean densitometric sum. In LF mice, approximately 73% of immunodetectable amyloid-β colocated with apo B, but in SFA mice this was significantly increased ($p < 0.05$) to nearly 87% (Table 1, columns 3 and 4). Figure 2 shows the extent of colocalisation in three dimensions of amyloid-β relative to apo B under high magnification.

To explore if abundance of the amyloid-β was interdependent with TRL biogenesis and secretion, correlation analysis with apo B was determined within enterocytes and in plasma respectively. Pearson's correlation analysis found that just 2% of amyloid-β and apo B fluorescent intensities were positively associated in enterocytes of LF or any of the SFA fed mice (table 1). Similarly, in plasma there was no evidence that the principal isoforms of amy-

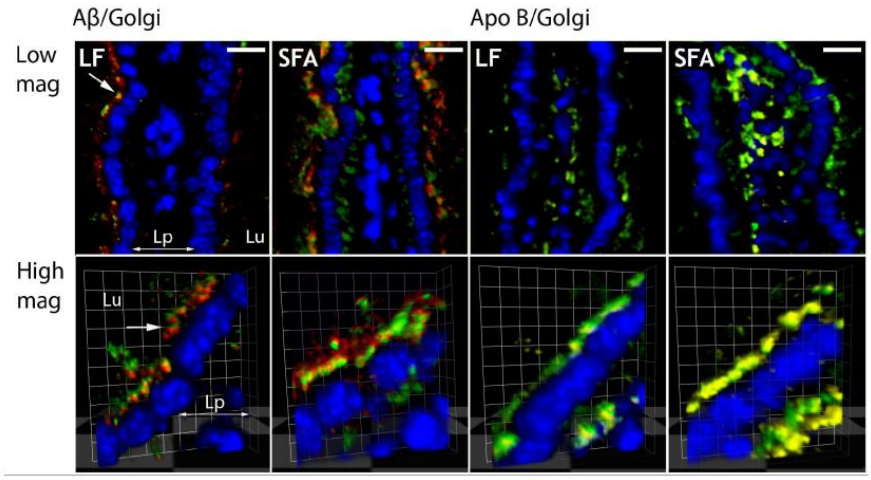


Figure 1
Enterocytic amyloid-β and apolipoprotein B colocalizes with Golgi-apparatus under LF and SFA feeding. The images depict the colocalization of Golgi-apparatus with amyloid-β (columns 1 and 2) and apo B (columns 3 and 4) in low-fat (LF) and saturated fat (SFA) fed mice. The upper row shows small intestinal villi at low magnification (mag) in two dimension, whilst the lower frames depicts enterocytes at high magnification in three dimensions. Amyloid-β as indicated in red, apo B as yellow, Golgi-apparatus as green, and nuclei as blue pixels. Where overlap of pixels occurs between amyloid-β (red) and Golgi-apparatus (green), an orange colour prevails. Similarly, the colocalization of apo B (yellow) with Golgi-apparatus (green) generates lime colour. Perinuclear (white arrow) and lamina propria (Lp) presence of amyloid-β and respective proteins are shown. Lu labels the lumen that represents the apical surface of the cell and Lp (lamina propria) is the direction of lacteals where lipoproteins are expelled via exocytosis. Scale: bar (2D images) = 10 μm; grid (3D images) = 3.63 μm.

loid-β (amyloid-β40 and 42) were associated with intestinal or hepatic apo B lipoproteins (figure 3).

Discussion

In this study the distribution and abundance of amyloid-β and apo B were detected in small intestinal enterocytes using an established double-labeled avidin-biotin IF microscopy technique [18]. Amyloid-β and chylomicron-apo B were remarkably colocalized in enterocytes, consistent with release of amyloid-β as a lipoprotein complex [7]. We also confirm that chronic consumption of SFA increases enterocytic amyloid-β and now show that this occurrence is concomitant with a substantially greater abundance of enterocytic apo B [6]. However, there was no evidence from this study that the biogenesis of amyloid-β and apo B are inter-dependent based on Pearson's correlation analysis within enterocytes and in plasma.

The biosynthesis of chylomicrons occurs in a multi-step process that requires the progressive lipidation of apo B an obligatory structural component of primordial lipoproteins secreted by the small intestine [20,21]. A number of proteins are reported to associate with nascent chylomicrons prior to secretion, including apo A-I, A-IV, apo J, apo D, apo E and small molecular weight proteins such as apo C-II. Nascent chylomicrons are then transported via the Golgi-apparatus to the basolateral membrane and secreted into lymphatics. The results from this study suggest that amyloid-β is secreted from small intestinal enterocytes as an apolipoprotein of chylomicrons.

Immunoreactivity for amyloid-β and apo B was found selectively within the ER/Golgi-apparatus and not on the plasma membrane. The findings are consistent with biogenesis of amyloid-β at the ER and translocation to primordial lipoproteins, rather than as a consequence of β APP processing. Similar results in hepatocyte cultures with secretion of amyloid-β also occurring exclusively as a lipoprotein complex [7].

Dietary SFA promote chylomicron biogenesis by stimulating apo B lipidation [22,23], an essential step to avoid post-translational degradation by intracellular proteases [24]. Greater lipid substrate availability (as a result of SFA ingestion) reduces the proportion of apo B that would otherwise be degraded. The SFA dietary intervention used in this study essentially doubled enterocytic apo B and a similar increase in amyloid-β abundance was observed.

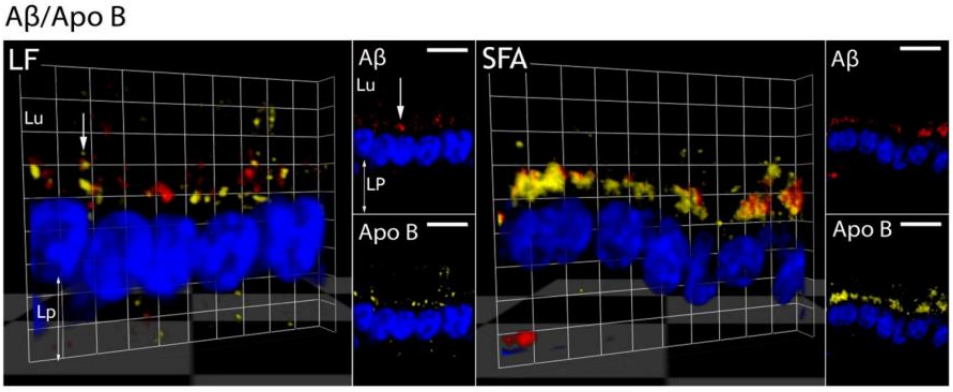


Figure 2
Enterocytic colocalization of amyloid-β with apo B under LF and SFA feeding. The enterocytic colocalization of amyloid-β (Aβ) with apolipoprotein B (apo B) in low-fat (LF) and saturated fat (SFA) fed mice is shown in three dimensions. The inset images depict the separate channel view for Aβ and apo B respectively. Amyloid-β is seen in red pixels, apo B as yellow and nuclei as blue. The perinuclear region (white arrow) and lacteal (Lp) orientation of enterocytes is indicated. Lu labels the lumen that represents the apical surface of the cell and Lp (lamina propria) is the direction of lacteals where lipoproteins are expelled via exocytosis. Scale: bar (2D inset images) = 10 μm; grid (3D images) = 3.63 μm.

However, the mechanisms by which SFA stimulate amyloid-β abundance and association with nascent chylomicrons are less clear. Saturated-FA may have a broader non-specific effect on enterocytic protein synthesis and consistent with the possibility of substrate driven biogenesis, Patil (2006) [25] found in neurons treated with palmitic acid resulted in increased upregulation BACE, a key enzyme complex involved in the processing of β *APP*. Alternatively, amyloid-β is an amphiphatic protein with a C-terminal domain that avidly binds with negatively charged hydrophobic lipids [15]. Increased substrate availability and synergistic lipidation of amyloid-β and apo B may promote the incorporation of amyloid-β into nascent chylomicrons and subsequently stimulate further synthesis of the proteins.

The SFA induction and secretion of enterocytic amyloid-β may be important in the context of AD risk. Recent studies suggest that blood-to-brain delivery of amyloid-β may contribute to amyloidosis, particularly when the concentration of circulating amyloid-β is chronically elevated [26-28]. This study suggest that SFA's increase synthesis and secretion of TRL associated amyloid-β concomitant with deterioration in blood-brain barrier integrity [29]. Indeed, the hypothesis is supported by studies in transgenic mice that over-express amyloid-β. In β APP/presenilin 1 transgenic mice, the plasma concentration correlated with secretion rates into blood of TRL's, which was increased 3-8 fold above wild-type mice [27]. Moreover, there was a positive association between plasma TRL-amyloid-β secretion with onset of cerebrovascular and parenchymal amyloidosis [29].

Conclusion

In this study, evidence in vivo that amyloid-β is secreted as a chylomicron complex and is stimulated by dietary SFA's is presented. Exploring this phenomenon in the context of plasma amyloid-β homeostasis and lipoprotein kinetics may provide insight into the putative association of high-fat diet with AD risk.

Table 1: Effect of SFA feeding on concentration and colocalization of enterocytic amyloid-β with apo B.

	Apo B*		Amyloid-β*		Overlap Coefficient		Pearson's Coefficient	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
LF	7013	790	5403	404	0.730	0.033	0.020	0.027
SFA	15840^	1812	13224^	1002	0.872^	0.022	0.015	0.023

*Mean enterocytic pixels value is expressed as mean densitometric sum and standard error of mean (SEM).
^Statistical significance was observed between LF and SFA groups with a p-value of at least less than 5%.

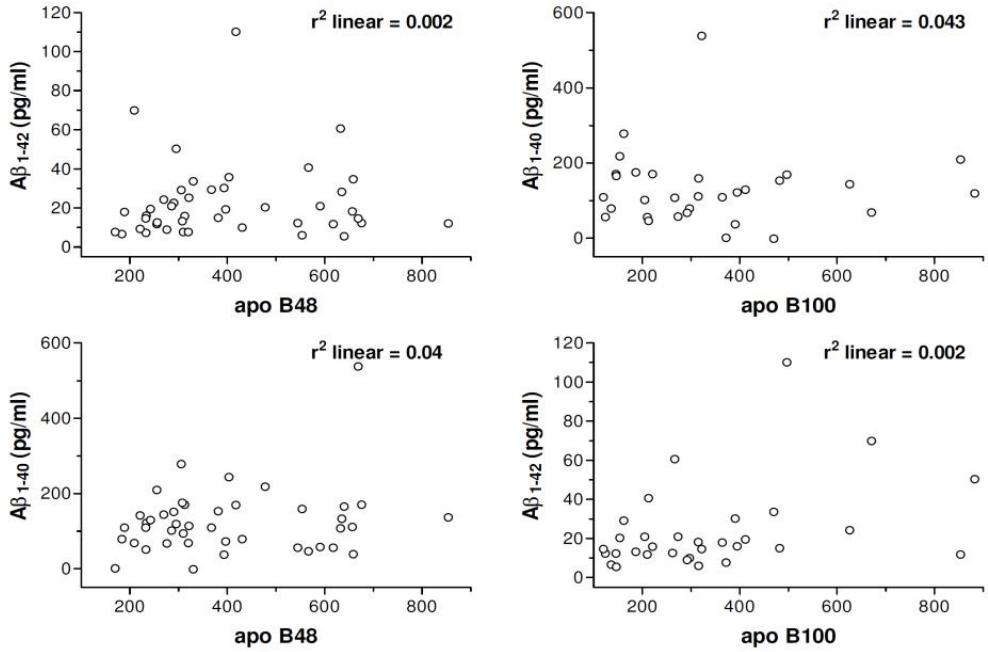


Figure 3
Correlation analysis of plasma amyloid-β40/42 with plasma apo B48/100. Correlation coefficients were determined with Pearson's correlation analysis where no outliers were identified.

List of Abbreviations

AD: Alzheimer's disease; apo: apolipoprotein; βAPP: β-amyloid precursor protein; IF: immunofluorescence; LF: low-fat; OC: overlap coefficient; PBS: phosphate buffered saline; SFA: saturated-fatty-acid; TRL: triglyceride-rich-lipoprotein

Competing interests

The authors acknowledge that there is no conflict of interest of any prior publication of any materials presented herein. All authors have seen and support the publication of this manuscript.

Authors' contributions

SG carried out the design of project, collection of data, immunofluorescence, statistical analysis and drafting of the manuscript. RT and MP-G assisted in the collection of tissues, interpretation of data and critically analyzing the manuscript content. SD helped in the statistical analysis of data and critically analyzing the manuscript content. JM conceived the study, helped in the interpretation of data, drafting of the manuscript, acquiring funding and role in general supervision of the research group. All authors have approved submission of the manuscript.

Acknowledgements

This project was supported by research grants awarded by the Australian Technology Network, Centre for Metabolic Fitness and the National Health and Medical Research Council of Australia.

References

1. Glenner GG, Wong CW: **Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein.** *Biochem Biophys Res Commun* 1984, **120(3):**885-890.
2. Park IH, Hwang EM, Hong HS, Boo JH, Oh SS, Lee J, Jung MW, Bang OY, Kim SU, Mook-Jung I: **Lovastatin enhances Abeta production and senile plaque deposition in female Tg2576 mice.** *Neurobiol Aging* 2003, **24(5):**637-643.
3. Runz H, Rietdorf J, Tomic I, de Bernard M, Beyreuther K, Pepperkok R, Hartmann T: **Inhibition of intracellular cholesterol transport alters presenilin localization and amyloid precursor protein processing in neuronal cells.** *J Neurosci* 2002, **22(5):**1679-1689.
4. Pallebage-Gamarallage MM, Galloway S, Johnsen R, Jian L, Dhaliwal S, Mamo JC: **The effect of exogenous cholesterol and lipid-modulating agents on enterocytic amyloid-beta abundance.** *Br J Nutr* 2009, **101(3):**340-347.
5. Simons K, Ikonen E: **How cells handle cholesterol.** *Science* 2000, **290(5497):**1721-1726.
6. Galloway S, Jian L, Johnsen R, Chew S, Mamo JC: **beta-amyloid or its precursor protein is found in epithelial cells of the small intestine and is stimulated by high-fat feeding.** *J Nutr Biochem* 2007, **18(4):**279-284.
7. Koudinov AR, Koudinova NV: **Alzheimer's soluble amyloid beta protein is secreted by HepG2 cells as an apolipoprotein.** *Cell Biol Int* 1997, **21(5):**265-271.
8. Mamo JC, Jian L, James AP, Flicker L, Esselmann H, Wiltfang J: **Plasma lipoprotein beta-amyloid in subjects with Alzheimer's dis-**

ease or mild cognitive impairment. *Ann Clin Biochem* 2008, **45(Pt 4):**395-403.
9. Boyt AA, Taddei K, Hallmayer J, Mamo J, Helmerhorst E, Gandy SE, Martins RN: **Relationship between lipid metabolism and amyloid precursor protein and apolipoprotein E.** *Alzheimer's Reports* 1999, **2:**339-346.
10. James AP, Pal S, Gennat HC, Vine DF, Mamo JCL: **The incorporation and metabolism of amyloid-beta into chylomicron-like lipid emulsions.** *J Alzheimers Dis* 2003, **5:**179-188.
11. Golde TE, Eckman CB: **Cholesterol modulation as an emerging strategy for the treatment of Alzheimer's disease.** *Drug Discov Today* 2001, **6(20):**1049-1055.
12. Koudinov A, Matsubara E, Frangione B, Ghiso J: **The soluble form of Alzheimer's amyloid beta protein is complexed to high density lipoprotein 3 and very high density lipoprotein in normal human plasma.** *Biochem Biophys Res Commun* 1994, **205(2):**1164-1171.
13. Zlokovic BV, Martel CL, Mackic JB, Matsubara E, Wisniewski T, McComb JG, Frangione B, Ghiso J: **Brain uptake of circulating apolipoproteins J and E complexed to Alzheimer's amyloid beta.** *Biochem Biophys Res Commun* 1994, **205(2):**1431-1437.
14. LaDu MJ, Pederson TM, Frail DE, Reardon CA, Getz GS, Falduto MT: **Purification of apolipoprotein E attenuates isoform-specific binding to beta-amyloid.** *J Biol Chem* 1995, **270(16):**9039-9042.
15. Shao H, Jao S-C, Ma K, Zagoriski MG: **Solution Structures of Michelle-bound Amyloid beta1-40 and bet1-42 Peptides of Alzheimer's Disease.** *J Mol Biol* 1999, **285:**755-773.
16. Galloway S, Pallebage-Gamarallage MM, Takechi R, Jian L, Johnsen RD, Dhaliwal SS, Mamo JC: **Synergistic effects of high fat feeding and apolipoprotein E deletion on enterocytic amyloid-beta abundance.** *Lipids Health Dis* 2008, **7:**15.
17. Nelson TJ, Alkon DL: **Protection against beta-amyloid-induced apoptosis by peptides interacting with beta-amyloid.** *J Biol Chem* 2007, **282(43):**31238-31249.
18. Takechi R, Galloway S, Pallebage-Gamarallage MM, Johnsen RD, Mamo JC: **Three-dimensional immunofluorescent double labelling using polyclonal antibodies derived from the same species: enterocytic colocalization of chylomicrons with Golgi apparatus.** *Histochem Cell Biol* 2008, **129(6):**779-784.
19. Manders EMM, Verbeek FJ, Atenm JA: **Measurement of co-localization of objects in dual-colour confocal images.** *J Microsc* 1993, **169(3):**375-382.
20. Hussain MM, Kedees MH, Singh K, Athar H, Jamali NZ: **Signposts in the assembly of chylomicrons.** *Front Biosci* 2001, **6:**D320-331.
21. van Greevenbroek MM, de Bruin TW: **Chylomicron synthesis by intestinal cells in vitro and in vivo.** *Atherosclerosis* 1998, **141(Suppl 1):**S9-16.
22. Davidson NO, Kollmer ME, Glickman RM: **Apolipoprotein B synthesis in rat small intestine: regulation by dietary triglyceride and biliary lipid.** *J Lipid Res* 1986, **27(1):**30-39.
23. Green PH, Riley JW: **Lipid absorption and intestinal lipoprotein formation.** *Aust N Z J Med* 1981, **11(1):**84-90.
24. Lee DM, Singh S: **Degradation of apolipoprotein B-100 in human chylomicrons.** *Biochim Biophys Acta* 1988, **960(2):**148-156.
25. Patil S, Sheng L, Masserang A, Chan C: **Palmitic acid-treated astrocytes induce BACE1 upregulation and accumulation of C-terminal fragment of APP in primary cortical neurons.** *Neurosci Lett* 2006, **406(1-2):**55-59.
26. LaRue B, Hogg E, Sagare A, Jovanovic S, Maness L, Maurer C, Deane R, Zlokovic BV: **Method for measurement of the blood-brain barrier permeability in the perfused mouse brain: application to amyloid-beta peptide in wild type and Alzheimer's Tg2576 mice.** *J Neurosci Methods* 2004, **138(1-2):**233-242.
27. Burgess BL, McIsaac SA, Naus KE, Chan JY, Tansley GH, Yang J, Miao F, Ross CJ, van Eck M, Hayden MR, *et al.*: **Elevated plasma triglyceride levels precede amyloid deposition in Alzheimer's disease mouse models with abundant A beta in plasma.** *Neurobiol Dis* 2006, **24(1):**114-127.
28. Takechi R, Galloway S, Pallebage-Gamarallage MMS, Wellington CL, Johnsen RD, Dhaliwal SS, Mamo JCL: **Differential effects of dietary fatty acids on the cerebral distribution of plasma derived apo B lipoproteins with amyloid-beta.** *Br J Nutr* 2009 in press.
29. Takechi R, Galloway S, Pallebage-Gamarallage M, Wellington C, Johnsen R, Mamo JC: **Three-dimensional colocalization analysis of plasma-derived apolipoprotein B with amyloid plaques in APP/PS1 transgenic mice.** *Histochem Cell Biol* 2009, **131(5):**661-666.

CHAPTER 5

Chapter 5: Effect of dietary fatty acids on enterocytic beta-amyloid abundance

5.1 Introduction and background:

5.1.1 Global variation in Alzheimer's disease prevalence

Global variations in diet and lifestyle and in particular dietary fat intake have been linked to the incidence and prevalence of AD (Grant 1998). Geographically, AD is not uniform and there is a significantly lower prevalence in developing countries in comparison to developed countries (Prince et al. 2004, Chandra et al. 1998, Hendrie et al. 1995). A meta-study (Ferri et al. 2005) shows that AD incidence is highest in North and Latin America (5.4 %), followed by Europe (4.4 %) and developed pacific regions including Australia, Japan, Singapore and South Korea (4.3 %). Developing west pacific regions (China and Vietnam (4 %)), Africa (3.6 %), Indonesia (2.7 %), Thailand (2.7 %), Sri Lanka (2.7 %) and India (1.9 %) have lower AD prevalence.

Countries with a high incidence of AD generally have higher fat intake than countries exhibiting a low incidence of AD. Moreover, accelerated rates of prevalence of AD in developing countries are thought to reflect in part, a transition to western diet and lifestyle patterns. The Honolulu-Asia Aging study showed that when Japanese men migrated to countries with greater dietary saturated fat intake (than Japan), these men were at greater risk of developing AD (Shadlen et al. 2000, Havlik et al. 2000, Graves et al. 1999).

5.1.2 Fatty acid types differentially regulate risk of developing Alzheimer's disease

Although general trends are suggestive that total fat and caloric intake are positively correlated with increased prevalence of AD, epidemiological studies are consistent with the notion that the AD-dietary fat nexus principally reflects fat-type. This hypothesis is perhaps not surprising given that the significant body of literature

demonstrating an association between dietary SFAs intake and chronic vascular-disorders such as cardiovascular disease (CVD), diabetes, obesity and AD (Morris et al. 2003). Conversely, a Mediterranean diet rich in olive oil (MUFA-rich) and 'oily fish' rich in n-3 PUFAs can be protective against AD (Scarmeas et al. 2007, Scarmeas et al. 2009).

5.1.3 Saturated fatty acids and cholesterol in Alzheimer's disease risk

A number of epidemiological studies show that high dietary intake of SFAs and cholesterol is linked with increased prevalence of AD. A longitudinal cohort study of 1449 subject based CAIDE (Cardiovascular risk factors, Aging and Dementia) study with median follow-up time of 21 years, showed that dietary SFAs during midlife was associated with poorer cognitive function and mild-cognitive impairment (Eskelinen et al. 2008). Similarly, another study of 815 subjects aged 65 and over with 4 year follow-up showed that intake of SFAs was positively associated with developing AD (Morris et al. 2003). In the Rotterdam Study of 5386 subjects over the age of 55, increased SFAs and cholesterol intake was were linked to increased risk for dementia after just a 2 year follow-up (Kalmijn et al. 1997) and in another large epidemiological study, Luchsinger et al. (2002) also reported that high intake of SFAs increase the risk of AD. Dietary cholesterol and high serum cholesterol levels mid-life may have synergistic effects with SFAs for increasing AD risk based on observations reported by Kivipelto et al. (2001, 2002).

5.1.4 Unsaturated fatty acids in Alzheimer's disease risk

Studies of dietary behaviour in the United States of America (Scarmeas et al. 2007, Feart et al. 2009), France (Tangney et al. 2011), South Italy (Solfrizzi et al. 1999), and more recently, Australia (Gardener et al. 2012) suggest that increased intake of MUFAs can be protective against cognitive decline and by extension AD. In addition, a number of epidemiological studies show that frequent consumption of oily fish has been shown to be protective against cardiovascular disease, cognitive decline and AD (Barberger-Gateau et al. 2002, Morris et al. 2003, Morris et al. 2006, Freund-Levi et al. 2006, Schaefer et al. 2006; Kalmijn et al. 1997, van Gelder et al. 2007). Oily fish is a major source of n-3 PUFAs, mainly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), both of which are important for brain

structure and function. Docosahexaenoic acid improves learning ability in animals (Lim and Suzuki 2000, Gamoh et al. 2001, Calon et al. 2004) and DHA has also been shown to decrease Aβ secretion from neuronal cells (Lukiw et al. 2005). As the body can only synthesise a small amount of EPA and DHA from α-linolenic acid (ALA) (Pawlosky et al. 2001), dietary intake of long chain n-3 fatty acids forms the major brain source. Despite these findings that support the benefits of PUFAs in dementia prevention, other studies have shown no benefit of fish intake on dementia risk (Laurin et al. 2003, Engelhart et al. 2002). Devore et al. (2009) attributes the discrepancy between such findings to be a result of the different stages of AD progression, heterogeneity in risk factors and complexity of the pathophysiological evolution of the disorder.

5.1.5 Vascular changes and origin of beta-amyloid in Alzheimer's disease pathology

A significant body of research has investigated factors regulating central biosynthesis, secretion and oligomerisation of cerebral Aβ. However, the exact origin of Aβ deposits in amyloid plaque or precursor cerebral amyloid angiopathy is presently not established. Since plaque and capillary deposits of Aβ are homologous to soluble Aβ found in blood and CSF (Busciglio et al. 1993, Vigo-Pelfery et al. 1993), the exact source/s of cerebral insoluble Aβ cannot be distinguished. Therefore, it is reasonable to assume brain Aβ can be derived from peripheral sources in addition to the central nervous system per se. There are several lines of evidence that support the notion that peripheral (blood-derived) Aβ may contribute to cerebral Aβ pathology (Zlokovic et al. 1993). Clifford et al. (2007) showed that 19 out of 21 AD brains showed extravasation of plasma proteins and in the same study, venous injection of fluorescence labelled Aβ into BBB damaged mice (induced by pertussis toxin) showed leakage of Aβ40 and Aβ42 in brain arterioles and parenchyma. Perfusion of synthetic Aβ40 and Aβ42 in neck blood vessels of guinea pigs has also been shown to cause BBB sequestration and transport of Aβ40 and Aβ42 into the brain (Martel et al. 1996). There is evidence that soluble Aβ travels bilaterally from the blood to the brain via non-specific and active pathways and via specific transporters, carriers or receptors and that plasma soluble Aβ can contribute to cerebral pathology (Zlokovic 2005) (refer to Chapter 1, section 3.0).

5.1.6 High fat diets; lipogenic organs and Alzheimer's disease risk

The functional units of lipogenic organs, the small intestine (Galloway et al. 2007, Pallebage-Gamarallage et al. 2009) and liver (Koudinov and Koudinova 1997, Galloway et al. Unpublished observations) can synthesize and secrete hydrophobic Aβ complexed to lipoproteins. Galloway et al. (2009) reported that absorptive epithelial cells of the small intestine showed increased enterocytic abundance of Aβ in response to a well tolerated diet that was enriched in SFA and cholesterol. Conversely, fasting abolished enterocytic Aβ suggesting a postprandial lipid regulatory pathway of enterocytic Aβ secretion (Galloway et al. 2007). Consistent with these findings, Boyt et al. (1999) reported increases in soluble APP following an oral mixed meal challenge containing significant dietary lipids. Moreover, James et al. (2003) showed that Aβ can bind and is metabolised when complexed with triglyceride-rich lipoproteins. These findings are consistent with the notion that lipoprotein-Aβ production and secretion into plasma is a postprandial phenomenon and are the first in the literature to suggest novel and alternative mechanisms of action concerning dietary fat induced increases in peripheral Aβ-lipoproteins post meals.

In a physiological context, metabolism of postprandial lipoproteins has been linked to initiation of an inflammatory cascade of events in atherosclerosis and arterial entrapment of apo B-containing lipoproteins (including CMs) in the intimal arterial wall (Proctor et al. 2003). Interestingly, in cases of probable AD and cognitive impairment, subjects also have exhibited delayed responses to oral fat loading test. Mamo et al. (2008) suggested that late-onset AD could in part be consequence of delayed apo B-Aβ-lipoprotein clearance. Indeed, plasma Aβ has been shown to negatively affect the integrity of the BBB by damaging endothelial and smooth muscle cells resulting in altered blood flow and increased vascular resistance (Thomas et al. 1996, Crawford et al. 1998, Arendash et al. 1999, Suo et al. 1998, Jancso et al. 1998). These findings led to the hypothesis that exaggerated vascular exposure to post-prandial lipoprotein Aβ may compromise cerebral capillary function with subsequent parenchymal extravasation of blood-derived apo B-lipoprotein Aβ. This hypothesis is consistent with previous findings on intestinally and hepatically derived apo B in cerebral amyloid plaques (Namba et al. 1992).

Although the differential regulation of SFAs (Galloway et al. 1997) and cholesterol (Pallebage-Gamarallage et al. 2009) has been previously reported, the effect of UFAs in enterocytic Aβ has not been previously studied. As dietary fats can differentially regulate metabolism of exogenously derived lipids by modulating the production and secretion of intestinal apo B-containing lipoproteins, they can by extension also regulate the apo B-Aβ-lipoprotein complex which is implicated in AD. A primary objective of this candidacy was to determine the effects of diets containing predominantly SFA, MUFA or PUFA on enterocytic Aβ abundance.

The objective of this study is to investigate if fatty acid types (saturated fat, monounsaturated fat or polyunsaturated fat) can differentially regulate intestinal Aβ expression and plasma abundance of apo B and Aβ. We hypothesize that saturated fat will increase, whilst unsaturated fatty acids will decrease enterocytic Aβ. To test the hypothesis, we used wild type mice fed different either low 4 % w/w fat diet or 20 % w/w diet as predominant SFA, MUFA or PUFA.

5.2 Materials and methods

5.2.1 Animals and maintenance

Six week-old female wild-type C57BL/6J mice weighing approximately 16g were obtained from the Animal Resources Centre (ARC, Perth, Western Australia). Animals were randomly divided into groups (n=12 per group): LF (low fat), SFA (saturated fatty acid), MUFA (monounsaturated fatty acid) or PUFA (polyunsaturated fatty acid) group. Animals were contained in an environment which was controlled for temperature, air pressure and lighting (12:12 h light/dark cycles). Mice had access to food and water *ad-libitum* and remained on diets for periods of 19 w and 38 w, the latter to explore potential duration effects. The body weight of mice and the amount of food and water ingested were recorded weekly to ensure adequate food consumption and growth. Procedures relating to mice handling and sacrifice were performed in accordance with the Animal Ethics Committee Guidelines and

were approved by a National Health and Medical Research Council (NH&MRC) of Australia accredited ethics committee (Curtin University ethics approval no. R 02-07).

5.2.2 Dietary intervention in C57BL6J mice

Experimental diets were manufactured by Specialty Feeds (Glenn Forest, Perth, Western Australia) and were supplied in 5 kilogram vacuum sealed (under nitrogen) air tight bags. Standard low fat (LF) rodent laboratory chow (AIN93M, Specialty Feeds, Western Australia) contained 4 % fat (w/w) and high fat (HF) chow contained 20.3 % (w/w) (or 40 % digestible energy) as fat. The low fat diet consisted of 4 % (w/w) total fat of which 2.4 % was oleic acid (18:1 n-9). High fat (HF) diets were made by replacing canola oil of LF chow with cocoa butter (SFA group), Sunola oil (MUFA group) and NUMEGA fish oil (PUFA group). The SFA chow (SF07-050, Specialty Feeds, Western Australia) predominantly contained 5.16 % palmitic acid (16:0) and 7.31 % stearic acid (18:0) and also 6.62 % monounsaturated fat as oleic acid. The MUFA chow (SF07-051, Specialty Feeds, Western Australia) contained 15.7 % oleic acid, 2.4 % linoleic acid (18:2 n6) and trace amounts of other fats. The PUFA chow (SF07-049, Specialty Feeds, Western Australia) was enriched with fish oil and contained 8.2 % docosahexaenoic acid (DHA 22:6 n3), 2.0 % eicosapentaemoic acid (EPA 20:5 n3) with a high n-3/n-6 ratio of 13.4. The PUFA diet also contained 3.26 % palmitic acid and 2.25 % oleic acid (refer to Table 1 for fatty acid composition for each diet).

Table 1: Fatty acid composition of dietary groups (given as % of total weight)

Diet	LF	SFA	MUFA	PUFA
Fat added	Canola oil	Cocoa butter	Sunola oil	NUMEGA fish oil
Total fat	4	20.30	20.30	20.30
SFA	**0.30**	**12.99**	**1.74**	**5.12**
Saturated fatty acids C12 and less	n/a	0.1	not detected	n/a
Myristic acid (14:0)	trace	0.05	0.02	0.54
Pentadecanoic acid (15:0)	n/a	0.01	n/a	0.16
Palmitc acid (16:0)	0.2	5.16	0.85	3.26
Magaric acid (17:0)	n/a	0.05	n/a	0.18
Stearic acid (18:0)	0.1	7.31	0.87	0.92
Arachidic acid (20:0)	n/a	0.24	n/a	0.06
Behenic acid (22:0)	n/a	0.04	n/a	n/a
Tetracosanoic acid (24:0)	n/a	0.03	n/a	n/a
MUFA	**2.4**	**6.69**	**15.79**	**3.19**
Palmitoleic acid (16:1)	n/a	0.05	0.02	0.66
Heptadecenoic acid (17:1)	n/a	0.01	n/a	0.1
Oleic acid (18:1 n-9)	2.4	6.62	15.7	2.25
Gadenoic acid (20:1)	n/a	0.01	0.07	0.18
PUFA	**1.2**	**0.72**	**2.83**	**11.38**
n-6/n-3 ratio	1.20	13.40	12.48	0.07
Linoleic acid (18:2n-6)	0.8	0.67	2.42	0.23
a-linoleic acid (18:3n-3)	n/a	0.05	0.13	0.09
y-linoleic acid (18:3n-6)	0.4	not detected	n/a	0.08
Stearidonic acid (18:4n-3)	n/a	n/a	0.08	n/a
Arachidonic acid (20:4n-6)	trace	not detected	0.2	0.46
EPA (20:5n-3)	trace	not detected	not detected	2
DPA(22:5n-3)	n/a	not detected	not detected	0.3
DHA (22:7n-3)	trace	not detected	not detected	8.22

n/a, data not available

Table 1 summarizes fatty acid compositions of chow diet of each group: LF, SFA, MUFA and PUFA. Proteins, carbohydrates, minerals and vitamins were comparable between groups.

LF low-fat, *SFA* saturated fatty acid, *MUFA* monounsaturated fatty acid, *PUFA* polyunsaturated fatty acid.

5.2.3 Sample collection

Mice from each dietary group were randomly selected for sacrifice at 19 w and the remainder were sacrificed at 38 w. Mice were anaesthetized with an intraperitoneal injection of Phenobarbital (45 mg/kg). Blood and tissue collection methods were as described previously (Galloway et al. 2007, Pallebage-Gamallage et al. 2009). Briefly, blood was collected by cardiac puncture into ethylene-diamine-tetracetic acid (EDTA)-tubes in ice. For intestinal Aβ immunofluorescence, the digestive tract was removed and small intestinal length measured from the pyloric sphincter to ileocecal valve and recorded. Thereafter the length of the small intestines were flushed with chilled phosphate buffered saline (PBS, pH=7.4) and 2 cm was cut and removed for fixation in 4 % paraformaldehyde. Tissues were fixed for 24 h and processed and longitudinal sections were embedded into paraffin wax blocks. Sections were trimmed to where all villi were exposed. Five-micron serial sections were cut and mounted on silanised-coated slides for immunostaining and histology.

5.2.4 Plasma beta-amyloid analysis

Plasma Aβ was measured using a commercially available Biosource ELISA kits which detects mouse Aβ40 (KMB3481; Invitrogen, Carlsbad, CA USA) and Aβ42 (KMB3441; Invitrogen, Carlsbad, CA USA) with rabbit monoclonal antibody raised C-terminus of mouse Aβ42. ELISA immunodetection method was performed as per the instructions of the manufacturer and as previously described (Takechi et al. 2010). The standard provided for $A\beta_{40}$ is detectable from 0 – 500 pg/ml and Aβ42 detection range is from 0-200 pg/ml. Manufacturers claim Aβ40 antibody does not recognise Aβ42 or Aβ43 and Aβ42 antibody does not detect Aβ40 or Aβ43.

5.2.5 Plasma lipid analysis

Plasma lipids were measured using absorbance based assays which are commercially available. Total plasma triglycerides were determined by measurement of surrogate marker glycerol produced from triglyceride hydrolysis (TR 1697, Randox laboratories, U.K). Cholesterol esterase/oxidase technique was used to determine total cholesterol measurements (CH 201, Randox laboratories, U.K).

5.2.6 Plasma apolipoprotein B analysis

Plasma apo B was determined in mouse plasma by Western blots and quantified by densitometry as previously described (Galloway et al. 2009).

5.2.7 Immunofluorescent detection of enterocytic beta-amyloid

Analysis of intestinal Aβ using immunofluorescent microscopy was done as previously described (Takechi et al. 2008b, Pallebage-Gamarallage et al. 2012). In brief, tissue sections (5 µm thick) were deparaffinised and rehydrated and then placed in boiling deionised water for 15 mins to retrieve antigens and for a further 10 mins in PBS to permeabolise tissues before blocking in 20 % goat serum. Rabbit anti-human Aβ (AB5076, Chemicon Temecula, CA) was diluted to 1:1000 in PBS and then added to slides and allowed to incubate overnight at 4 °C followed by 1 h incubation in goat anti-rabbit IgG (1:200) (E0432, DAKO, Carpentaria, CA) at room temperature. Immunofluorescence was visualised with addition of anti-rabbit IgG with Alexa488 (1:100). Cell nuclei were labelled with DAPI (1:1000) (Invitrogen, Victoria, Australia) and sections were mounted on anti-fade mounting medium and sealed with a transparent hardening lacquer.

5.2.8 Imaging

Immunofluorescent digital images of X 100 magnification (Plan Neofluar x20 objective, 1.3 numerical aperture) for photomicroscopy were acquired by AxioCam HRc camera (Zeiss, Germany) using AxioVert 200 M inverted microscope (Zeiss, Germany). Identical conditions were used to simultaneously capture fluorescent images. Assessment of pixel intensity for Aβ measurements were done using version 5.5 of AxioVision software. Filters used for excitation and emission were determined based on individual characteristics of fluorophores. Filter 38 (Ex BP470/40, beam splitter FT495 and Em BP525/50) and 49 (Ex G365, beam splitter FT395 and Em BP445/50) were used selectively for Alexa488 and DAPI stains respectively. Detection of emission from Alexa488 did not overlap fluorescent emission from DAPI staining. In addition, images were analysed in a darkroom and therefore detection of Aβ fluorescence was free from other external light sources.

5.2.9 Quantification of intestinal beta-amyloid abundance

Quantification of fluorescence was performed as previously described (Pallebage-Gamarallage et al. 2012). In brief, approximately 40-50 2-dimensional (1388 x 1040 pixels) images were captured per treatment group with each image containing 4 or more villi. The densitometric sum of Aβ was calculated and villus nuclei were counted for each image using automatic measurement tool in Measurement module (AxioVision software version 4.7.1, Carl Zeiss, Germany). For each image, hue, saturation and threshold was standardised and image modification was limited only to removal of auto fluorescent debris of intestinal lumen. Quantification of enterocytic Aβ was determined by analysis of overall pixel intensity (densitometry) and dividing by the total number of cells counted per field of view.

5.2.10 Statistical analysis

The effect of HF feeding on Aβ abundance, body weight gain, plasma Aβ and plasma lipids were assessed by univariate analysis. Outliers were removed using Post-hoc comparisons of means were performed and if equal variance was not found, then Mann-Whitney test was used to compare the difference between individual groups. Correlation studies were performed using Pearson's correlation if data was considered normal; otherwise, Spearman's correlation was used. A P-value < 0.05 was considered a statistically significant. Data was analysed and graphs were generated using GraphPad Prism version 6, Microsoft Excel 2007 and SPSS version 17.0. If data was not considered normally distributed (column statistics tool, GraphPad Prism), then data was presented with histogram showing min to max; alternatively, bar graph exhibiting mean plus standard error of mean (S.E.M) was used.

5.3 Results

5.3.1 Body weight and plasma lipids

Body weight and plasma lipids were determined to monitor animal wellbeing and tolerance to diets. All mice gained weight consistently each week throughout the course of experiment. There were significant increases in body weight in all treatment groups at 19 and 38 w compared to initial weight at the commencement of dietary regimen (Fig. 1). At the end of the experiment (38 w), the MUFA group gained significantly greater weight than other treatment groups. Conversely, the PUFA treated mice gained significantly less weight than other fat supplemented treatment groups at the end of the dietary regimen (Fig. 1).

Despite a more modest weight gain in mice maintained on a PUFA enriched diet, these mice were moderately hypercholesterolemic compared to control mice maintained on the LF chow diet. The mean plasma cholesterol concentration in mice maintained on an SFA or MUFA enriched diet was approximately 25 % greater than the LF-treated mice. Plasma triglycerides were not significantly different between different treatment groups.

In mice, the liver produces apo B48 in addition to apo B100 so there is no equivocal marker of intestinal versus hepatic TRLs (Greeve et al. 1993). Therefore, total apo B-containing lipoproteins were determined by Western blot analysis. In the C57BL/6J mice used in this study and consistent with the plasma lipid data suggesting that the diets were well tolerated, chronic maintenance of mice on diets enriched in either SFA, MUFA or PUFA had no significant effect on the plasma concentration of plasma apo B following 19 or 38 w of feeding (Fig. 2 pooled data is shown).

Figure 1: The effect of dietary fats on body weight and weight gain in wild-type C57BL6J mice

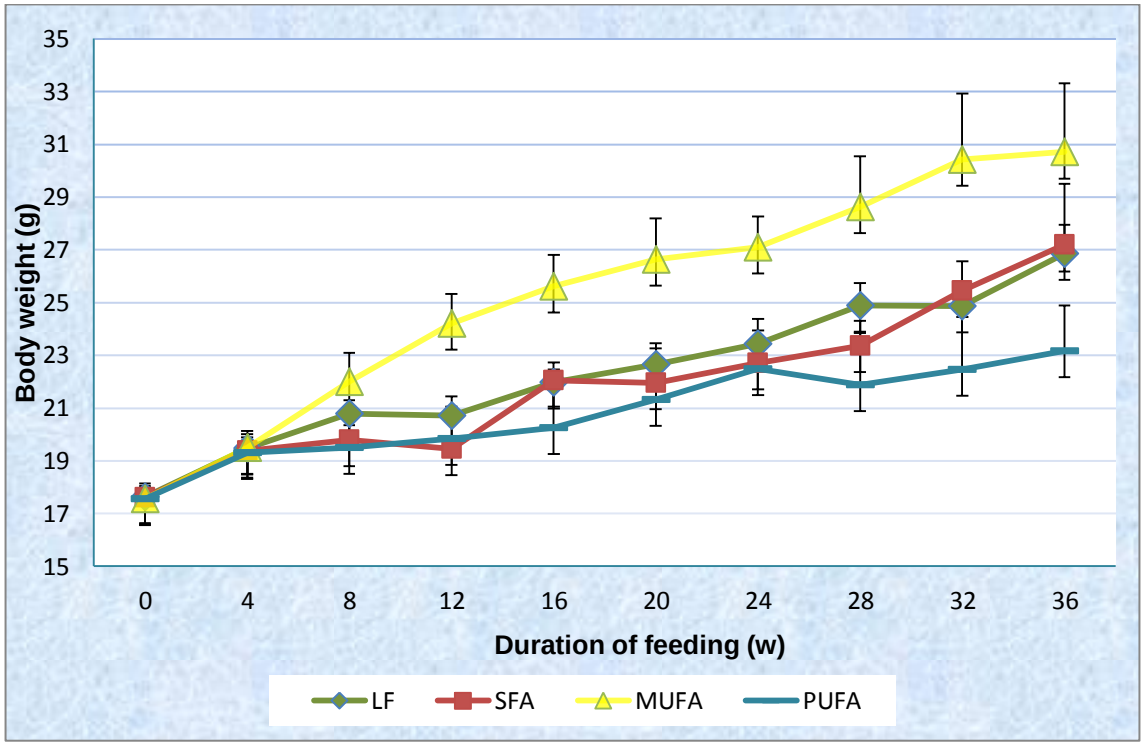


Figure 1 Data indicated as mean body weight ± standard error of the mean (S.E.M). Change in mean body weight of mice maintained on LF, SFA, MUFA and PUFA diets were recorded over the duration of the experiment where 0 weeks indicate the commencement of feeding. Weight of mice (all groups) was significantly higher than commencement weight (week 0).

LF low-fat, *SFA* saturated fatty acid, *MUFA* monounsaturated fatty acid, *PUFA* polyunsaturated fatty acid.

Table 2: The effect of various dietary fats on plasma lipid concentration in wild-type C57BL6J mice

	Cholesterol (mM)	Triglyceride (mM)
LF	1.84 ± 0.075	0.64 ± 0.056
SFA	2.23 ± 0.274	0.72 ± 0.125
MUFA	2.30 ± 0.312	0.67 ± 0.095
PUFA	2.57 ± 0.164	0.74 ± 0.105

Table 2 Data indicated as mean ± standard error of mean. Univariate ANOVA test was used to determine differences between plasma lipids and dietary groups. Single-factor ANOVA shows no difference between plasma cholesterol and triglyceride between groups at 19 weeks.

LF low-fat, *SFA* saturated fatty acid, *MUFA* monounsaturated fatty acid, *PUFA* polyunsaturated fatty acid.

Figure 2: The effect of various dietary fats on plasma apolipoprotein B concentration in wild type C57BL6J mice

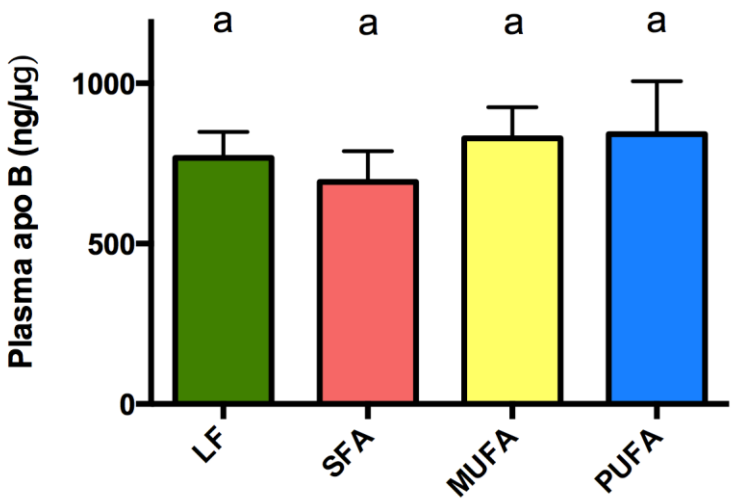


Figure 2 Histogram for plasma concentrations of total apolipoprotein B (Fig. 2) indicated as mean ± standard error of mean. [a] No significant difference was determined between dietary LF, SFA, MUFA and PUFA groups ($P < 0.05$).

LF low-fat, *SFA* saturated fatty acid, *MUFA* monounsaturated fatty acid, *PUFA* polyunsaturated fatty acid.

5.3.2 The effect of duration of feeding on intestinal abundance of beta-amyloid in C57BL6J mice

The abundance of intestinal Aβ in response to dietary intake of various fatty acids in C57BL6J wild-type mice was assessed with immunofluorescent microscopy and protein abundance quantified by calculating fluorescent pixel intensity per cell nuclei (Fig. 4). We confirm previous studies that Aβ immunofluorescence (yellow) was evident within the mucosal layer of the duodenum comprised principally of non-ciliated columnar epithelial cells or enterocytes and lamina propria. The distribution of Aβ along the villi is more prominent on the body and tips of villi compared to basal located and crypt villi cells. Within enterocytes, the location of Aβ is consistent with intracellular transport of synthesized proteins from Golgi apparatus and endoplasmic reticulum towards secretion on basolateral surface in polarised enterocytes. In addition, Aβ was visible particularly within the capillaries and lacteals of the lamina propria as evidence of the secretory pathway.

The overall effects of diets enriched in fatty acids (all fatty acid treatment groups combined) compared to LF-controls on enterocytic-specific beta amyloid. Enterocytic Aβ was increased approximately 6-fold in the fat-supplemented mice compared to mice maintained on the LF diet (Fig. 3: LF versus HF). Duration of feeding effects was considered to exclude potential confounding effects of dietary acclimatization. Without discrimination of fatty acid treatment types, the data suggests sustained amplification effects over a 38 w intervention period of fatty acid dietary enrichment on enterocytic abundance of Aβ compared to LF fed controls (Fig. 3). A comparison of 38 w fed versus 19 w control mice did however suggest the possibility of a modest age-associated effect on enterocytic Aβ; however, the magnitude of this putative age-associated effect was relatively small compared to the effects of fat supplementation per se.

Figure 3 shows HF feeding significantly increased enterocytic abundance with no distinguishable difference between 19 w and 38 w durations. Similarly, the LF feeding showed no difference between both durations. Based on the lack of difference between the 19 w and 38 w durations, the data was pooled (Fig. 4).

Figure 3. Effect of duration of feeding on enterocytic beta-amyloid abundance

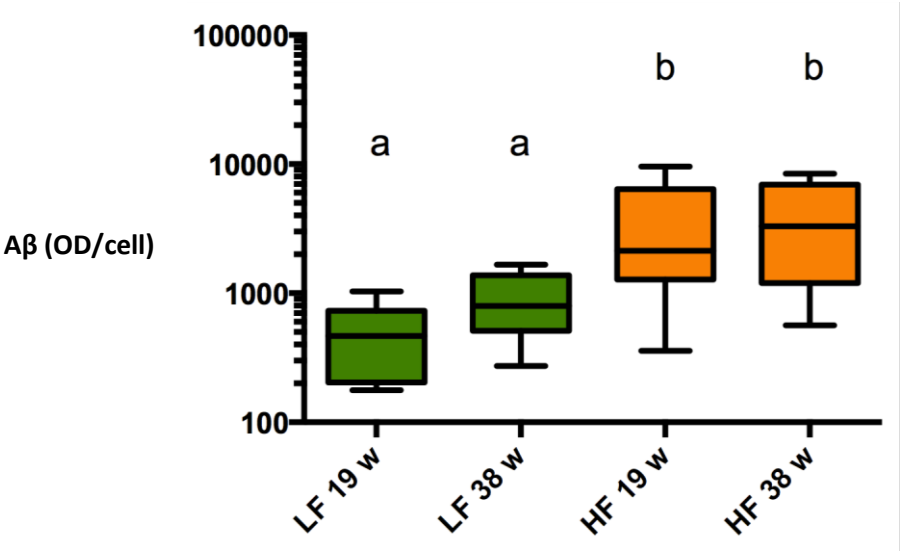


Figure 3 Quantitative analysis of enterocytic Aβ between low fat (LF) and high fat (HF: SFA + MUFA + PUFA) diets and comparison of enterocytic Aβ expression at 19 w and 38 w of feeding. Data is indicated as histogram with min, max, median, quartiles. [a] [b] Increased duration of feeding did not significantly increase the expression of enterocytic Aβ. High fat feeding significantly increased the expression of enterocytic Aβ compared to LF for 19 w and 38 w using ($P < 0.05$)

LF low-fat, *SFA* saturated fatty acid, *MUFA* monounsaturated fatty acid, *PUFA* polyunsaturated fatty acid. OD optical density.

5.3.3 The effect of dietary fats on intestinal abundance of beta-amyloid in C57BL6J mice

The pattern of Aβ fluorescence was detected along the villi and in the perinuclear location of all dietary groups; however, the abundance of Aβ was different between groups. All HF fed groups (SFA, MUFA and PUFA) exhibit significant increase in enterocytic Aβ (pixel area of staining and pixel intensity per cell) compared to LF fed controls (Fig. 4). High magnification depicts Aβ is abundant within the perinuclear location and traces of Aβ is also evident in the basolateral location of enterocytes under SFA feeding (Fig. 4a: SFA frame). In comparison to SFA group, unsaturated fatty acid (UFA) feeding showed a similar pattern of Aβ immunofluorescence within the absorptive villi of the small intestine. Both MUFA and PUFA had relatively greater abundance of Aβ immunofluorescence in perinuclear and basolateral areas of enterocytes (Fig. 4a: MUFA and PUFA frames).

5.3.4 Quantitative evaluation of enterocytic beta-amyloid and fatty acid effects

Equal caloric diets composed of 20.3 % (w/w) of fats were incorporated into the low fat chow containing 4 % (w/w) of fats. The SFA diet contained 65 % total SFAs (predominantly palmitic and stearic) significantly enhanced enterocyte expression of Aβ by 3.5-fold in comparison to LF group (Fig. 4b). In comparison, feeding of 77 % is oleic acid (MUFA) and 56 % n-3 PUFAs also increased intensity of Aβ immunofluorescence by 5.4-fold and 7.5-fold respectively compared to LF (Fig. 4b). Quantitative evaluation shows that fatty acid and increase calorie intake from fat significantly increase enterocytic Aβ abundance.

Figure 4a: The effect of dietary fatty acids on beta-amyloid abundance in small intestinal villi

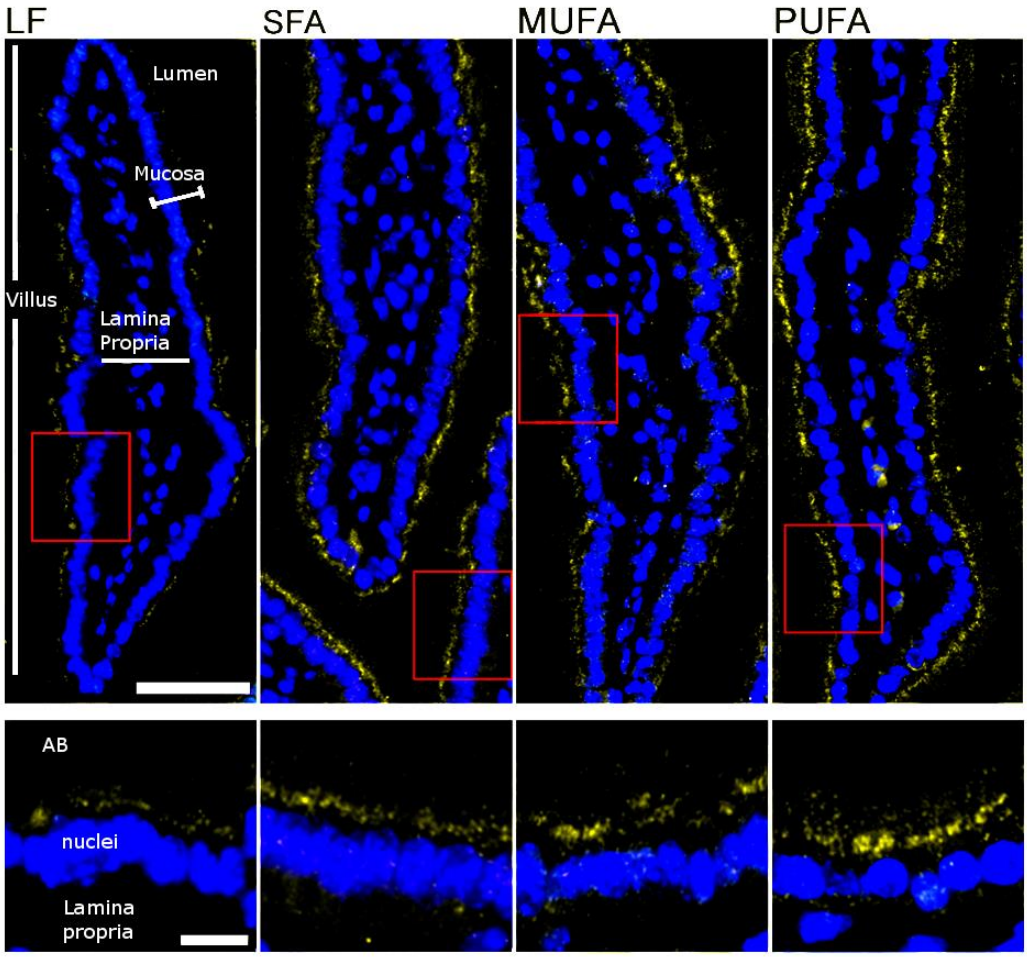


Fig. 4a Immunofluorescent images representative for each group showing small intestinal Aβ (yellow) and enterocyte cell nuclei (blue). Top row of images shows low-magnification and bottom row shows magnified portion of villi (red box) indicating cellular abundance of Aβ in enterocytes. The villus consists of a single outer layer of which the predominant cell type is enterocytes (mucosa). The lamina propria form the centre of the villi and consist of lacteals, blood vessels, lymphatics, and connective tissue. Presence of Aβ is concentrated in the perinuclear region of enterocytes with some Aβ evident towards the basal location of cells and within the lacteals. The pattern of staining is consistent in each group. Abundance of Aβ is increased in high fat (HF) feeding which was specific to the type of fat ingested. Scale bar = 40 µm (top row) 10 µm (bottom row).

LF low fat, *SFA* saturated fatty acid, *MUFA* monounsaturated fatty acid, *PUFA* polyunsaturated fatty acid.

Figure 4b: Quantification and analysis of the effect of dietary fatty acids on enterocytic beta-amyloid.

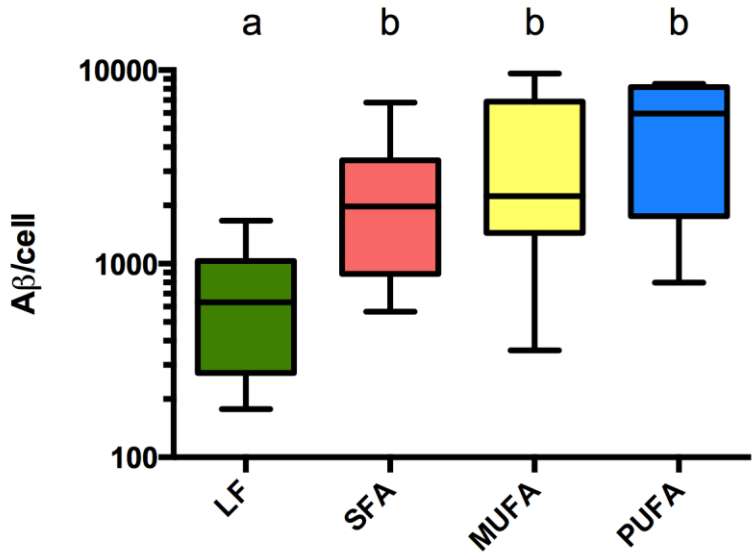


Figure 4b bar graph and table presents histogram of median with min, max and quartiles for optical density value of enterocytic Aβ immunofluorescence between LF control, SFA, MUFA and PUFA groups. Optical density per cell = [mean OD x pixel area] / total number of cells counted. [b] Indicates significant difference compared to [a] LF group ($P < 0.05$).

LF low fat, *SFA* saturated fatty acid, *MUFA* monounsaturated fatty acid, *PUFA* polyunsaturated fatty acid.

5.3.5 Effect of dietary fats on plasma beta-amyloid 40 and beta-amyloid 42

Increased enterocytic abundance of Aβ may translate to increased secretion into lacteals and blood. Plasma Aβ40 and Aβ42 were measured and having confirmed no duration-of-feeding effects within treatments (19 w versus 38 w), therefore pooled data is presented. Irrespective of treatment group, there was approximately an 8-fold greater concentration of Aβ40 in plasma compared to Aβ42. The potential differential effects of dietary fat supplementation on plasma Aβ isoforms are indicated in Figure 5. There was no substantive effect of dietary fatty-acid treatment on plasma Aβ40 concentration compared to LF-controls. However, the median plasma Aβ42 appeared markedly greater in fat supplemented mice with MUFA > PUFA > SFA > LF. However, significant within fatty-acid treatment group variability did not realize statistically significant differences (Fig. 5a and 5b). Consistent with the notion of greater Aβ42 relative to Aβ40, the plasma ratio was significantly greater in mice on fatty acid enriched diets compared to LF-controls (Fig. 5c).

Figure 5a: The effect of dietary fats on plasma beta-amyloid 40

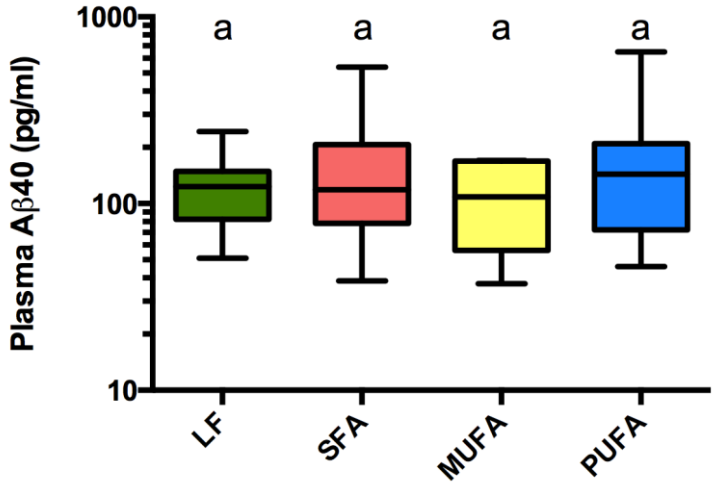


Figure 5b: The effect of dietary fats on plasma beta-amyloid 42

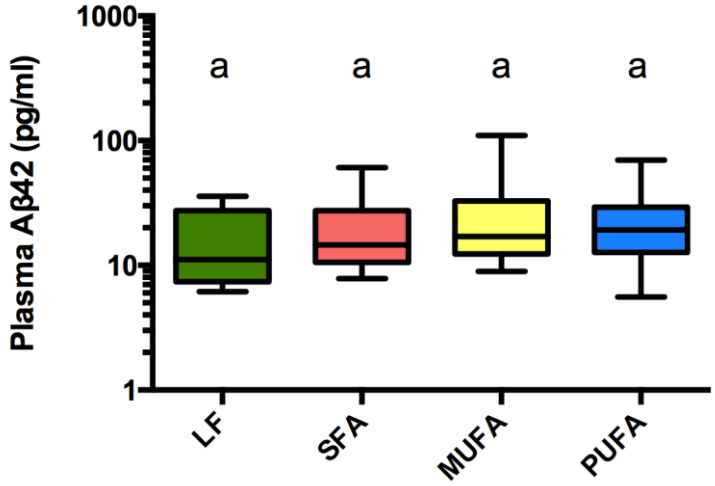


Figure 5c: The effect of dietary fats on plasma beta-amyloid 42/40 ratio

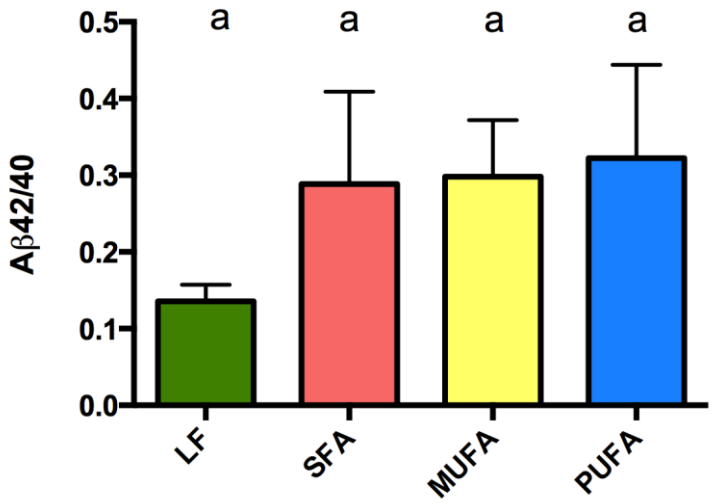


Figure 5 Data shows plasma Aβ40 (5a), Aβ42 (5b) and Aβ42/Aβ40 ratio (5c) measurements for LF, SFA, MUFA and PUFA groups. Plasma Aβ40 and Aβ42 are indicated by histogram (median, min, max and quartiles) and Aβ42/40 ratios are indicated as mean ± standard error of mean [a] No significant difference was found between dietary groups (P > 0.05).

LF low fat, *SFA* saturated fatty acid, *MUFA* monounsaturated fatty acid, *PUFA* polyunsaturated fatty acid.

5.3.6 Association between plasma beta-amyloid and plasma apolipoprotein B

Figure 4 in this chapter indicates that diets enriched in dietary TG enhance Aβ abundance within enterocytes and this observation was independent of the TG fatty-acid isoforms of SFA, MUFA and PUFA. Chapter 4 (Galloway et al. 2009) demonstrated the colocalisation of enterocytic apo B with Aβ and degree of association determined by Manders Overlap Coefficient was significantly enhanced in SFA fed group. These observations are consistent with the possibility of increased chylomicron biogenesis and secretion occuring in association with increased enterocytic Aβ abundance, although the abundance of Aβ and apo B were not dependent (chapter 4) suggesting that the relative abundance of Aβ per CM may also differ between dietary treatment groups. The relative ratio of plasma Aβ to apo B lipoproteins may therefore provide some insight into relative enrichment as a consequence of fatty acid feeding. Figure 6a shows no significant difference between fatty acid treatment groups in the concentration of Aβ40 relative to the LF group, however there was significant inter-group variability and therefore statistical significance was not realized. Some evidence of increased enrichment with Aβ42 of TRLs within the SFA and PUFA groups is however indicated (Fig. 6b).

Figure 6a: fatty acid diet effects on ratios of beta-amyloid 40 to apolipoprotein B

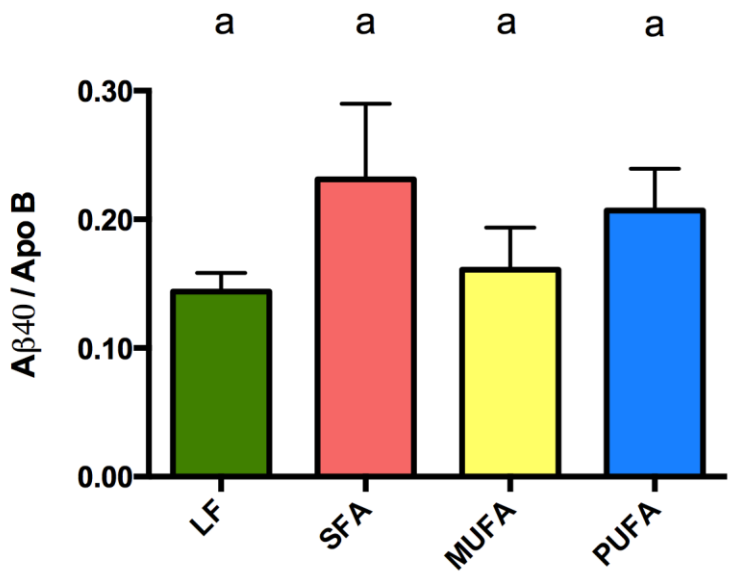


Figure 6b: fatty acid diet effects on ratios of beta-amyloid 42 to apolipoprotein B

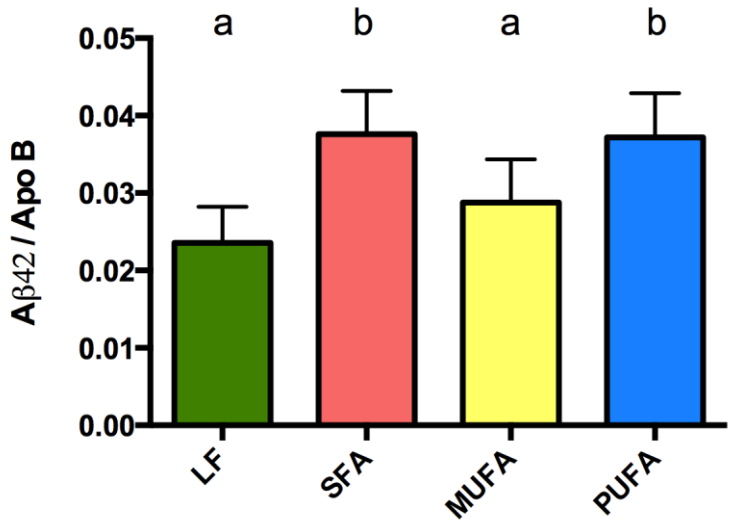


Figure 6 Ratios of Aβ40 and Aβ42 to plasma apo B are shown as histograms in figure 7a and 7b respectively. [a] Indicates significant difference compared to LF control group ($P < 0.05$).

LF low fat, *SFA* saturated fatty acid, *MUFA* monounsaturated fatty acid, *PUFA* polyunsaturated fatty acid.

5.4 Discussion

This study utilized wild-type C57BL/6J mice that are widely used for studying lipid disorders such as atherosclerosis. Experimentally, these mice have demonstrated good tolerance to high SFA and CH diets, with a similar degree of enrichment as indicated in this study (Pallebage-Gamarallage et al. 2009, Pallebage-Gamarallage et al. 2012). The aetiology of AD is unknown, however the presence of an isoform of apo E, E4 (allele) in up to 50 % of AD cases has led to the notion that AD could be a result of a disorder in lipid metabolism (Lindsay et al. 2002). However, some dietary fats especially SFAs have been increasingly reported to be associated with increased prevalence of AD in large multi-nation epidemiological studies (Grant 1998, Morris et al. 2003, Shadlen et al. 2000, Havlik et al. 2000, Graves et al. 1999). However, the link between AD and postprandial lipoproteins, including apo B-containing lipoproteins, which are produced and secreted in response to exogenous availability of dietary fatty acids is not well understood. We previously hypothesized that dietary fat induced stimulation of CM's enriched in Aβ, might increase AD risk as a consequence of exaggerated cerebrovascular exposure to lipoprotein associated Aβ (Takechi et al. 2008a). Consistent with this hypothesis, we reported that C57BL/6J mice maintained on an SFA diet for 12 w had BBB dysfunction and blood-to-brain delivery and retention of apo B associated lipoproteins (Takechi et al. 2010b). However, mice maintained on diets enriched with MUFA or PUFA did not demonstrate brain parenchymal extravasation of apo B lipoprotein associated Aβ. Therefore, a primary objective of this candidacy was to explore the hypothesis that diets enriched in saturated or unsaturated fatty acids, differentially modulate Aβ biogenesis.

Immunofluorescent microscopy captured images showed expression of Aβ within the proximal small intestine of C57BL/6J mice and specifically on the mucosal layer and confined to simple epithelium containing enterocytes as well as lacteals. Within polarized enterocytes, the presence of Aβ was visible within the perinuclear location, the site of ER and Golgi apparatus. The pattern and location of staining is consistent with previous demonstrations of intestinal Aβ abundance using IHC (Galloway et al. 2007, Galloway et al. 2008, Galloway et al. 2009, Pallebage-Gamarallage et al. 2009). The pattern of staining remained consistent across groups although staining intensity was significantly increased in all HF groups (Fig. 4a).

Quantification of enterocytic Aβ was done by image capture and measurement of fluorescent intensity or pixel density with automatic measurement software. Pixel density was then divided by the number of cells counted to determine mean Aβ intensity per cell. High SFA, MUFA and PUFA diets all led to significant increase in enterocytic abundance of Aβ compared to LF controls ($P < 0.05$). This data indicates that HF feeding increased enterocytic Aβ without discrimination for fatty acid treatment group. In contrast to the hypothesis suggesting that SFA specifically would increase enterocytic Aβ relative to equicaloric diets enriched in MUFA or PUFA, there was no difference on enterocytic Aβ between fatty-acid treatment groups per se. An increase in Aβ staining observed under HF feeding was evident within the perinuclear region of the cytosolic compartment suggesting direct effects on Aβ biogenesis.

The distribution of Aβ was primarily evident in the perinuclear region and not identified at the cell membrane. This observation is consistent with earlier findings that 90 % of APP is found to be present in the Golgi apparatus and the trans-Golgi network (Haass et al. 1992). In addition, the polarized enterocytes (columnar ciliated simple epithelial cells) are structurally built for absorption at the apical surface and secretion at the baso-lateral surface. One study of APP production in polarised kidney cells found that a large amount of sAPPα is produced in the trans-Golgi network (Haass et al. 1995) and a small amount on plasma membrane. Colocalization of Aβ with apo B within enterocytes (Chapter 4), is consistent with previous findings in cultured liver cells (HepG2 cells) where intracellular Aβ was recovered with components of lipoproteins including phospholipids, TG, CH (and CH esters) and apolipoproteins (Koudinov and Koudinova 1997). Like hepatocytes, it is conceivable that within enterocytes, Aβ is also processed in a similar manner. This led to the conclusion that enterocytic Aβ might be involved with the apo B lipidation process and CM maturation and thereafter secreted associated with CMs (Galloway et al. 2009).

The current chapter identified that both enterocytic Aβ and apo B can be strongly stimulated by dietary fatty acids, but as findings of Chapter 4 suggests, the synthesis of Aβ and apo B occur independently. Although literature on the effect of fatty acids on APP processing is limited, there are some studies of fatty acids effects on APP processing in cell culture models. According to Patil et al. (2006) SFAs

induced an increase in Aβ production by up regulate β-secretase processing of APP in primary rat cortical neurons and Lui et al. (2004) demonstrated that oleic acid treatment in PSwt1 CHO cells increases APP expression and Aβ abundance. However, Lim et al. (2005) showed that DHA up-regulates α-secretase and reduced β-secretase therefore reducing Aβ synthesis. It is possible that fatty acids may also modulate amyloidogenic or non-amyloidogenic processing of APP within the enterocytes.

The abundance of enterocytic Aβ could reflect a balance of production and section. A limited number of cell culture studies suggest that SFA increase whilst MUFA and PUFA decrease secretion of Aβ in neurons and Chinese hamster ovary cell culture medium compared to BSA controls (Oksman et al. 2006, Amtul et al. 2011b). Although these observations may provide some relevant details regarding the fatty acid effects on Aβ metabolism, we show secretion of Aβ occurs in association with apo B-lipoproteins and therefore findings cell culture models may be considered less physiologically relevant in context of current findings. An earlier study in our laboratory reported high-SFA feeding (with similar SFA content to this study) and 1 % CH for 32 d increased the intracellular abundance of Aβ in mouse enterocytes, however did not markedly increase lacteal abundance compared to controls (Pallebage-Gamarallage et al. 2012). Due to similarity in animal model and diet, it is reasonable to assume that SFA-induced increases in enterocytic Aβ in this current study is probably a consequence of heightened synthesis rather than decreased secretion.

The findings in chapter 4 and in other studies (James et al. 2003, Koudinov and Koudinova 1997) demonstrate that Aβ directly associates with lipids and apo B in Golgi and is secreted in a complex with lipoproteins. In cell culture studies using transformed colonic enterocytes (CaCo2 cells), the addition of SFA (palmitic acid), MUFA (oleic acid) and n-6 PUFA (linoleic acid) were shown to increase secretion of TG and apo B48 comparably (van Greevenbroek et al. 1996). In addition, the total number of secreted apo B molecules was doubled compared to controls, but did not differ between media of fatty acid treatment group per se (van Greevenbroek et al. 1996). The findings reported by van Greevenbroek et al. (1996) are broadly consistent with the primary findings reported here, in that enterocytic abundance of Aβ was increased to a similar extent in mice randomized to either of the fatty acid

treatment groups. However, the *in vivo* model utilized in this study found no evidence of accumulation of lipoprotein associated Aβ within plasma. In this study, the fat-enriched diets were well tolerated with no evidence of dyslipidemia. Hence, a notional increase in secretion of postprandial lipoprotein-Aβ as a consequence of fatty acid feeding, may have been accommodated within the ordinary metabolic pathway, that being hydrolysis by endothelial lipases and thereafter, clearance by high affinity (receptor-mediated) pathways. Chronic excessive ingestion of SFA ordinarily suppress apo B/E receptor abundance, the primary pathway by which apo B lipoproteins including CM-remnants are cleared (Dane-Stewart et al. 2003). However, there was no evidence of apo B lipoprotein accumulation in the SFA treated mice in this study.

The experimental design adopted is in the context of a post-prandial state so the time of sacrifice was restricted within a 2 h time frame following the ordinary feeding (dark room) feeding period. It is possible that variability in the absorptive state masked and an adequate clearance pathway masked a transient state of postprandial hyperamyloidemia. To consider this possibility directly, in vivo fat loading studies are required or alternatively collection of lymph via cannulation techniques. The latter however is difficult technique to achieve in murine models due to size.

In the absence of direct assessment of secretion, it is notionally also possible to assume that enterocytic accumulation of Aβ is indicative of reduced lipoprotein assembly and secretion into lacteals and lymph. However, this explanation is unlikely given a number of studies in vivo and in vitro which uniformally demonstrates that dietary fatty acids and in particular fatty acids stimulate assembly of large TRLs within enterocytes (Field et al. 1988, Williams et al. 2004, van Greevenbroek et al. 1996). In addition, the abundance of Aβ relative to apo B generally tended to be greater in the fat-treated mice compared to controls. The latter was particularly indicated for the Aβ42 isoform.

The relevance of these findings in the context of AD risk is unclear. The SFA diet utilized in this study has equivocally been demonstrated to compromise BBB integrity, resulting in brain parenchymal retention of lipoprotein associated-Aβ which may be inflammatory and exacerbate amyloidosis (Takechi et al. 2010a).

Compromised capillary integrity may be a consequence of increased exposure to post-prandial lipoprotein-Aβ; however frank accumulation of lipoproteins was not indicated in mice maintained on SFA treatment. On the other hand, there was evidence of increased enterocytic abundance of Aβ and apo B that may be indicative of heightened secretion during the absorptive phase. Rather, mice fed an equicaloric diet relative to SFA but enriched in MUFA or PUFA also were consistent with increased production of CM-associated Aβ. On the basis therefore that vascular exposure to lipoprotein-Aβ is not profoundly similar between fatty acid treatment groups, it would suggest that capillary dysfunction is a consequence of the fatty acid phenotype and not vascular exposure per se. Thereafter however, the metabolic lipoproteins cascade that occurs as a consequence of extracellular retention may be exacerbated as a consequence of the Aβ enrichment. If this were the case, the propensity of greater Aβ42 enrichment may be notable.

CHAPTER 6

Chapter 6: General discussion

This chapter will present a general discussion for the findings presented in this thesis. Chapter 2 identified the presence of Aβ within intestinal lipogenic enterocytes and in conjunction with chapter 3 and 5 showed that enterocytic Aβ abundance can be regulated by the ingestion of dietary fats. Chapter 3, 4 and 5 further explored the physiological implications of this finding in the context of metabolism of Aβ and production of postprandial lipoproteins derived from enterocytes. In this chapter, the findings will be considered principally in a physiological context; methodological considerations and possible future studies will also be discussed. Consideration of the findings in the broader context of AD risk will be considered and will include co-authored papers where significant contributions were made.

6.1 Enterocytic beta-amyloid homeostasis

6.1.1 Summary of enterocytic beta-amyloid findings

High fat feeding is associated with a number of metabolic diseases including cardiovascular disease, diabetes, hyperlipidemia, hypertension, stroke, obesity and cognitive decline. In recent years, fatty acids have been increasingly recognised in the modulation of appetite, hormone secretion, inflammatory, oxidative and insulin metabolic pathways (Ulven and Christiansen 2015). The effect of these pathways on intestinal Aβ is currently not known; however, since the intestines are the first to respond to the availability of fat from the intestines, it would be reasonable to assume that Aβ expression is a direct result of high fat intake. This is supported by cell culture studies which were mentioned in chapter 5 (Oksman et al. 2006, Amtul et al. 2011b, Patil et al. 2006, Lim et al. 2005).

This thesis was the first article to show the presence of Aβ within enterocytes and explore the relative expressions of Aβ in response to intake of dietary fats in murine model. Given that enterocytic Aβ expression is responsive to the intake of dietary fat or the availability of exogenous lipid substrate, it is reasonable to assume that it has a role in the metabolism of lipids, possibly as an apolipoprotein component of enterocytic lipoproteins.

This thesis presents the finding that Aβ, a protein which may accumulate in the brain over long periods of time and cause irreversible brain changes commonly reported in AD, is found in the absorptive enterocytes of the small intestine (chapter 2). Furthermore, the enterocytic expression of Aβ and apo B were sensitive to the exogenous availability of lipid substrate derived from dietary sources (chapters 3 and 4). Chapter 3 found that small intestinal villi height and overall intestinal length may be affected by dietary fat content in a manner which favours absorption, increasing the number of enterocytes. Chapter 4 further investigated the co-association of Aβ with apo B in the context of lipoprotein biogenesis and regulation by dietary fatty acids and CH. Contrary to the hypothesis indicated in the literature review, chapter 5 showed that enterocytic abundance of Aβ was not differentially regulated by SFA, MUFA and PUFA.

Enterocytic Aβ was predominantly localized with apo B within the perinuclear location of Golgi and ER of enterocytes indicated by significant co-localised immunodetection of Aβ and apo B. Although the different compositions of fatty acids (SFA, MUFA and PUFA) did not appear to differentially modulate enterocytic Aβ abundance, the calorie or total amount of fatty acid intake rather than saturation and chain length may be of greater importance with respect to promoting the biogenesis of Aβ. Our results were consistent with previous publications of reports that SFA, MUFA and PUFA equally promote apo B biogenesis and CM production (van Greevenbroek et al. 1996). These findings collectively support the concept that within enterocytes, Aβ is associated with postprandial apo B-containing lipoproteins (nascent CMs).

6.1.2 Role of small intestine adaptation in enterocytic beta-amyloid abundance

The absorptive enterocytes in the small intestine are responsible for the delivery and transport of 95 % of exogenous fats to the body. These specialised columnar epithelial cells absorb emulsified fats through the apical surface and secrete them into the lacteals as intestinal lipoprotein CMs via the basolateral surface. Following synthesis, CMs are secreted into mesenteric lymph and transported to thoracic duct from which they enter the circulation via the subclavian

vein. Circulating CMs are rapidly metabolised by lipoprotein lipase (LPL), which results in the formation of smaller TG-poor, CH and apo E rich CM remnants. These apo E-rich CM remnants are ordinarily rapidly cleared by the liver via receptor-specific processes (Hussain et al. 1996). Variation in dietary fat consumption and duration of feeding are proposed to alter dietary lipid absorption (Degrace et al. 1998) and enterocytic turnover (growth and proliferation) (Dauncey et al. 1983, King et al. 1983) as well as secretion rate and fatty acid composition of biliary CMs (Degrace et al. 1998). In addition, gastrointestinal transit time is increased after 14 d of HF feeding (Cunningham et al. 1991). Taken together, it is reasonable to assume the small intestine can play a significant role in the absorption dietary fats and secretion of plasma Aβ-apo B-lipoproteins.

Chapter 3 found that small intestinal villi height and overall intestinal length is influenced by habitual intake of dietary fat. In a physiological context, this dietary fat-induced adaptation in small intestinal physiology is favourable towards increasing absorption of dietary fat and secretion of Aβ-apo B-lipoproteins by increasing the total number of enterocytes. Pearson's correlation coefficient (chapter 4) shows that the presence of Aβ and apo B occur independently of each other, but both can associate during maturation of lipoproteins and thereafter be secreted as a lipoprotein complex. It is conceivable that the diet derived fatty acids can modulate APP processing pathways which are separate from the pathway which fatty acids affects apo B abundance. The role of fatty acids in APP processing and apo B will be discussed separately in this chapter.

6.1.3 Effect of fatty acids on amyloid precursor protein processing

Proteolytic processing of APP by α-secretase prevents the production of Aβ whereas processing by β- secretase (or BACE) and γ-secretase produces Aβ. Modulation of selective processing by inhibiting β- or γ- secretase has been regarded as a possible therapeutic target for reduction of Aβ that is pathologically implicated in AD (Nishitomi et al. 2006, Elvang et al. 2009). Results from chapter 5 showed no distinct changes in enterocytic Aβ in SFA, MUFA or PUFA diets. This however does not eliminate the possibility that enterocytic APP processing can be regulated by different fatty acid type in enterocytes and other cells. Although no other studies have researched the effect of dietary fats in enterocytic APP

processing per se, there are a number of studies in other cell types which offer insight into intracellular lipid regulation of APP processing (Lim et al. 2005, Patil et al. 2006, Parsons et al. 2007). Lim et al. (2005) found in the cortex of 17-19 month old transgenic Alzheimer mice models (Tg2576) fed a diet containing 0.6 % DHA, reduced insoluble amyloid plaques and specifically a reduction of Aβ42 within guanidine-soluble extracts. Further analysis showed that DHA specifically regulated APP levels by modulating preferential proteolysis by α-secretase by reducing β-secretase (Lim et al. 2005). These results are not consistent with our enterocytic data, which indirectly suggests a stimulatory effect of n-3 PUFA on APP processing. Moreover, in this study enterocytic isoforms of Aβ were not determined.

Further insight into the modulating effects of fatty acids on APP processing was gained by Patil et al. (2006) who showed in a transgenic Alzheimer's mouse model that palmitic acid treatment led to the accumulation of C-terminal fragment (CTF) in neurons via the upregulation of β-secretase within surrounding astrocytes (Patil et al. 2006). The function of β-secretase to cleave APP depends on its state of maturation. Indeed, Parsons et al. (2007) found inhibition of palmitoylation and farnesylation resulted in reduced dimerisation of β-secretase and lower levels of Aβ which indicates that β-secretase maturation is linked to intracellular modifications of fatty acids. Although β-secretase expression was not measured in this study, dietary fatty acids may differentially regulate enterocytic Aβ via altered β-secretase cleavage of APP.

6.1.4 Apolipoprotein E role in enterocytic triglyceride and beta-amyloid

In addition to the role in receptor uptake of TRLs, apo E is also responsible for production of VLDL in the liver. Low levels of apo E were linked to increased plasma concentrations of TRL's and cholesterol, which is due to inefficient clearance via apo E receptors (Quarfordt et al. 1995, Zhang et al. 1992, Kuipers et al. 1997). Studies also indicate that apo E deficiency also leads to significant hepatic accumulations in the liver on low-fat chow; more than halving TRL content in the liver. This was consistent with a more than trice reduction in rate of production of VLDL measured by triton WR 1339 intravenous injection (Mensenkamp et al. 1999). In the same study, cultured hepatocytes also showed a

significant decrease in intracellular TG in apo E KO mice; though a slight increase in TG was detected after 24 h incubation with 0.75 mM oleate. In addition, the rate of VLDL-TG production was directly proportional to the expression of apo E expression in the hepatocytes mRNA, leading to the conclusion that apo E associates with immature VLDL particles prior to secretion. In chapter 3, our results showed a slight but non-significant decrease in plasma TG in apo E KO mice either on low or high fat chow feed, but rather a significant increase in plasma CH. This is consistent with the plasma TG and CH levels measured in apo E KO mice by Mensenkamp et al. (1999).

The genetic expression of apo E has been shown in the gastrointestinal tract including the small intestine in humans. In the small intestine, apo E was specifically isolated in the macrophages, dendrite cells, and in lymph follicles of the lamina propria of mucosal layer (Niemi et al. 2002). There is some evidence that suggests apo E has a role in differentiation, proliferation, adhesion, structure, polarity and positioning of cells (Vogel et al. 1994, Lewalle et al. 1997, Handelmann et al. 1992, Scott et al. 1998). However, the study presented in chapter 3 contradicts these findings; apo E null mice shows increased the length/height of intestinal villi under low fat diet (compared to wild-type mice on LF diet) and decreased cells under high fat diet (compared to wild-type mice on HF diet). The diet-gene regulation of intestinal villi height/length and enterocytic cell number may indirectly influence the overall intestinal production of CMs and Aβ.

6.1.5 Enterocytic triglyceride and apolipoprotein B synthesis and secretion

Chapter 4 showed that enterocytic abundance of Aβ can occur independently from apo B; however, several studies support the binding and secretion of Aβ with apo B-containing lipoproteins from lipogenic cells (Koudinov and Koudinova 1997, James et al. 2003). In addition, secretion of surrogate maker sAPPα for Aβ proteolysis occur post intake of oral fat loading test, possibly from enterocyte source (Boyt et al. 1999). In blood, Aβ has high affinity to lipoproteins and apolipoproteins binding to more than 90 % Aβ (Koudinov et al. 1994, Stratman et al. 2005, Tokuda et al. 2000, Kuo et al. 1999, James and Mamo 2005). Taken together, these studies are supportive of the likelihood that Aβ binds to apo B-

lipoproteins in enterocytes prior secretion. The co-association of Aβ with perinuclear organelles indicate that Aβ is likely incorporated into CMs during maturation or lipidation stages of synthesis (discussed in detail in chapter 2). In addition, fatty acids irrespective of chain length and saturation can promote lipidation of apo B and drive synthesis and secretion of CMs. Since secretion of enterocytic Aβ was not measured in this study, in-situ isolation of immune-positive Aβ would reflect the balance of production and secretion of Aβ in complex with apo B-containing lipoproteins. Confirmation of Aβ secreted as a lipoprotein complex could be considered by immunoprecipitation of lymph.

The effect of fatty acids on secretion of apo B-lipoproteins can provide an indication of the effect of fatty acids on the secretion of Aβ-apo B-lipoproteins. Understanding the production and secretion dynamics of Aβ-apo B-lipoproteins within enterocytes is an important consideration in the interpretation of enterocytic Aβ results in chapters 2, 3, 4 and 5. A study of fatty acid effects in Caco-2 cells show that addition of high fat mixed-diet which contained predominantly either SFA, MUFA and n-6 PUFA for 24 h increased secretion of TG and apo B48 by 2-fold compared to controls, however there was no significant difference in secretion of apo B between different fatty acid groups (van Greevenbroek et al. 1996). Indeed one of our co-authored works also indicates that Aβ secretion remains unchanged between low fat and high fat feeding (Pallebage-Gamarallage et al. 2012). If indeed secretion rates are comparable across the different fatty acid groups and controls, this finding supports the observations made in chapters 2, 3, 4 and 5 that different fatty acids can indiscriminately drive greater Aβ production compared to a low fat diet within enterocytes.

6.1.6 Enterocytic and hepatic contribution to plasma beta-amyloid

Results in this thesis indicate that small intestinal enterocytes produce and secrete Aβ with apo B-lipoproteins. The pathological relevance and possibility that liver and small intestine can separately contribute Aβ-apo B48-lipoproteins and Aβ-apo B100-lipoproteins towards brain AD pathology has been discussed (Takechi et al. 2009). In brief, the roles of apo B48 and apo B100 have been previously studied in atherosclerotic plaques. Although both apo B48 and apo B100 have been found in endothelial cells and reside in the tunica intima/media of blood vessel walls (Proctor

et al. 2002, Olofsson and Boren 2005), apo B48 was found to be present in greater abundance (Pal et al. 2003). In addition, the increase in affinity of apo B48 to arterial wall was specific and not dependent on plasma concentrations (Pal et al. 2003). Both apo B48 and apo B100 can bind heparin sulphate proteoglycans (HSPGs) (Proctor and Mamo 2003), increased arterial retention of apo B48-lipoproteins can also extended to greater retention by extracellular matrices of the brain as shown by our recent study (Lam et al. 2011). In brief, this study explored the possibility that HSPGs, specifically biglycan, decorin and perlecan (not agrin) can facilitate the bindings of apo B to cerebral amyloid plaques of the brain (Lam et al. 2011). Currently, evidence suggests that intestinally derived Aβ contributes to greater plasma levels of apo B-containing lipoproteins postprandially; however, liver derived lipoproteins are present in greater concentrations in blood throughout the majority of the day. Therefore it is possible there can be differential effects of apo B100 and apo B48 Aβ containing lipoproteins may be relevant to risk for AD.

6.2 High fat diet and intestinally or hepatically derived beta-amyloid in Alzheimer's disease pathology

6.2.1 Dietary fat and blood-brain barrier damage: candidates contribution to co-authored manuscripts

Takechi et al. (2009) identified that apo B of small intestine and/or liver origin is present in cerebrovasculature and amyloid plaques. This study addresses the latter part of my broad hypothesis (see thesis introduction for hypothesis) and examines the putative effects of SFAs and UFAs dietary regimen on BBB integrity. This study directly extends on the findings of chapter 5 that enterocytic Aβ is enhanced under high-fat feeding, but demonstrates that SFA diets, not MUFA or PUFA, causes damage to BBB. In the article by Takechi et al, I contributed to care of animals, design of dietary intervention, sample collection, sample analysis and appraisal of draft manuscript. Secretion of plasma Aβ-lipoproteins from enterocytes may not be transported into the brain without demonstrable BBB damage and thus increased capillary permeability. Hence, the findings reported by Takechi et al were relevant in the context of risk for BBB-dysfunction.

The co-authored article titled “Dietary fats, cerebrovascular integrity and Alzheimer’s disease risk.” (Takechi et al. 2010a) addresses the broader hypothesis (Introduction and chapter structure section) in relation to the role of dietary SFA and lipoprotein-Aβ in cerebrovascular and amyloid plaque pathology. Beta-amyloid has “vasoactive” effects on the BBB including damage to endothelial cells (Thomas et al. 1997) and glial cells (George et al. 2004) resulting in a more permeable BBB. Chronic increases in plasma Aβ-lipoproteins induced by high dietary SFA intake could therefore damage cerebral blood vessels. One of the author’s own publications (Takechi et al. 2010b), using wild-type C57BL6J mice maintained on SFA feeding, compared to MUFA, PUFA and low-fat control diets showed marked leakage of the BBB, resulting in an increase in bi-directional transport of proteins across the BBB. Specifically, SFA feeding decreased expression of junction protein occludin along with increased influx of plasma apo B/IgG and increased efflux of S100β (Takechi et al. 2010b). Although blood to brain influx of Aβ-lipoproteins was not explored, cerebrovascular presence of apo B co-localised with Aβ indicates a high likelihood of Aβ-lipoprotein influx into the brain. In addition, colocalization of Aβ-apo B evident within cerebral blood vessels, proteoglycans of the cerebral matrices (Lam et al. 2011) and amyloid plaques suggests that intestinally or hepatically derived Aβ-lipoproteins can cross the BBB and bind to the brain via apo B binding ligands.

6.2.2 Summary of enterocyte or hepatocyte derived beta-amyloid in Alzheimer’s disease pathology

The results from chapter 5 did not indicate increased plasma Aβ as a result of dietary fat feeding (SFA, MUFA and PUFA). Based on these results it is unclear whether or not SFA-induced increases in Aβ-lipoprotein levels is causally associated with BBB dysfunction. Alternatively, SFA could damage BBB via other mechanisms such as lipotoxicity, oxidation, inflammation which has been summarized in detail elsewhere (see section 4 of the review article by Takechi et al. 2010a).

Takechi et al. (2010a) focuses on one of the key proteins which influences risk of developing AD, apo E4 and discusses the implications of apo E isoforms on TRL metabolism and related toxic mechanisms. Briefly, compared to other isoforms, apo E4 increases risk of developing AD by 17 % or 43 % dependant on whether an

individual contains one or two alleles (Strittmatter and Roses 1996). Apolipoprotein E4 could be implicated in AD pathology via by facilitating the blood to brain transport and parenchyma deposition of Aβ-lipoproteins. Apo E4 patients report “leaky” BBB compared to E2 and E3 subjects and under such circumstances this can lead to an increase in the influx of plasma Aβ-lipoproteins (Deane et al. 2008). Physiologically, apo E influences the metabolism of lipoproteins by binding to remnants (Hatters et al. 2006, Mahley 1988) and LDL receptor (Heeren et al. 2002, Krapp et al. 1996, Mamo et al. 1991). Compared to apo E3 and apo E2 which associates primarily with hepatically derived lipoproteins, apo E4 preferentially binds to CMs (Saito et al. 2003) and comprises up to 65 % of the protein component of CM-remnants (Campos et al. 1992). The presence of apo E on Aβ-apo B-lipoproteins can facilitate nucleation into brain matrices as apo E binds with high affinity to HSPGs. In addition, apo E is an important ligand for the binding of apo B-lipoprotein remnants to LRP1 which is implicated in cerebral Aβ efflux. However, cell culture studies indicate that the binding affinity of apo E isoforms to LRP is not different, indicating that efflux is relatively constant and may not be implicated in AD via this clearance route.

Cerebral Aβ-apo B-lipoproteins can deposit into the brain by association of apo B and various proteoglycans found in extracellular matrices of the brain. Proteoglycans, the predominant component of extracellular matrices can bind to both apo B and apo E (Flood et al. 2002, Bame et al. 1997). Studies have found HSPG, a type of proteoglycan, to be involved in the formation of amyloid plaques (van Horssen et al. 2003). Agrin, perlecan, biglycan and decorin are all proteoglycans which are capable of binding to apo B/apo E and these have been implicated in retention of lipoprotein (Iozzo 1998, Small et al. 1996, O’Brien et al. 1998, Olin et al. 2001). A recent co-authored in our laboratory (Lam et al. 2011), demonstrated using immune-based approach, the colocalization of Aβ-apo B with HSPGs in a transgenic (APP/PS1) mouse model of AD and found perlecan, biglycan and decorin to be co-localized with apo B and Aβ in amyloid plaques. This study provides a possible mechanism for the nucleation of CSF Aβ-apo B-lipoproteins to cerebral matrices.

6.3 Methodological considerations and study limitations

6.3.1 Immunohistochemistry and immunofluorescence for detection of beta-amyloid

As the detection of intestinal Aβ was crucial to our first article (chapter 2) *"B-amyloid or its precursor protein is found in epithelial cells of the small intestine and is stimulated by high fat feeding"* the results were dependent on the sensitivity and specificity of IHC method and in particular the specificity of the Aβ antibody to recognise Aβ antigen without binding to unwanted epitopes. Specific immunological methods were developed and employed to determine in-situ location of Aβ. As a negative control, antibody absorption method was used, which briefly involves pre-incubation of synthetic Aβ peptide with the primary Aβ antibody. Thereafter, the "absorbed" antibody was applied to the tissue as per IHC method and successful blocking or absence of staining signified that an antibody bound or absorbed Aβ. In addition to antibody pre-absorption, PBS was also used as a negative control with our experiments and transgenic mouse or human brain tissue with known amyloid plaques were used as positive controls. Antibody AB5076 was used for purposes of identification of intestinal Aβ in IHC and IF methods in subsequent experimental procedures (chapters 3, 4 and 5).

Immunohistochemistry is a useful technique in determining *in situ* location of antigens of interest. Immunohistochemistry is an increasingly used method for clinical and research applications and the number of publications using IHC has increased 3-fold in the past 20 years (Matos et al. 2010). Briefly, IHC is a straightforward method which allows *in-situ* isolation of specific antigens by colour-tagging which would otherwise remain undifferentiated by conventional histological techniques (Matos et al. 2010). By selective use of antibodies, IHC can be informative in recognising cellular or tissue responses *in vivo* by detecting changes in antigen expression (Brandtzaeg 1998). In addition, sensitivity of the methods can be adjusted by changing detection systems or decreasing antibody dilutions to enable recognition of very small amounts of antigen.

6.3.2 Quantification of enterocytic beta-amyloid

The second methodological hurdle in this candidacy was to determine a method for quantification for Aβ determined by IHC and IF. First of all, it was important that the chromatin or fluorescence visualised with IHC and IF respectively was representative of the relative amount of Aβ. A number of research papers have found correlations between antigen amount and IHC signal (Matkowskyj et al. 2003, True 1988). It is also conceivable that quantification of IHC and IF uses similar antigen-antibody methods such as ELISA technique and Western blotting which are widely accepted as quantitative (Matos et al. 2010).

Whilst IHC with semi-quantitative analysis were sufficient in determining the expression of intestinal Aβ, quantification of Aβ by IHC (chapter 2 and 3) was limited to manual methods of grading which is considered "semi-quantitative". Although analysis by visual identification of "chromatin" intensity predetermined by parameters and can be limited by observer bias and subjectivity (Shi et al. 1991, Shi et al. 1993, Mikaelian et al. 2004), this method is an accepted method for semi-quantification in IHC. Consideration of other factors in IHC, such as preservation of tissue and the ability of antibody to recognise the epitope, are vital to the process of quantification and has been discussed in detail by Walker (2006). In the methodologies used in this study, these conditions were tightly controlled between specimens that were prepared and treated under identical conditions of fixation, processing, cutting, staining and observed through identical optical chain.

Immunofluorescence was developed based on its more direct method of quantification from measurement of fluorescent intensity. In addition, IF was employed for its advantage of 3D double labelling of two or more antigens (objective in chapter 4). Therefore, the IF method was introduced in chapter 4 for advantages of digital image analysis with software which can selectively recognise and quantify the immunostaining results by calculating the density of pixels allowing for both co-localization and quantification. In chapter 4, the optical detectable "overlap" of two fluorescent intensities was used to determine the degree of co-association of cellular elements in-situ.

The objective of chapter 4 was to determine whether Aβ co localizes with intestinal apo B. In order to achieve this within tissues, a dual-labelling method using IF techniques was developed (for details, see Takechi et al. (2008b)). This

technique successfully overcomes the barriers of using polyclonal antibodies raised in the same species by diluting the first antibody and by the use of different secondary detection systems. In brief, this method used a low dilution of rabbit polyclonal apo B antibody (1:4000) adding anti-rabbit IgG with biotin to bind and thus block most of the apo B antigen site. Subsequently, the second antibody rabbit polyclonal anti-Golgi at dilution 1:10 was used to attach to the Golgi protein of interest and finally, secondary anti-rabbit IgG conjugated with FITC and avidin conjugated with Alexa 546 was added. This method allows for double labelling of 2 distinct polyclonal antibodies raised in the same species without cross-reactivity. Subsequent detection of Aβ was achieved without overlap of fluorescence emission from other fluorophores.

6.3.3. Recommendations for future work

Although the main objectives of the study have been thoroughly explored by the experiments conducted, there have been several limitations and therefore there expose possibilities for future studies. Extensions of this study may include 1) determining if different types of fats modulate the amyloidogenic processing of Aβ in hepatocytes and enterocytes; 2) modulate the binding or association of Aβ isoforms to chylomicrons and VLDL in response to fat intake; 3) Elucidate if absorption of triglycerides, and secretion, transport and clearance of CMs can be influenced by the presence of Aβ; 4) determine if intracellular Aβ interacts with fatty acids and play a role in incorporation into chylomicrons; and lastly, to explore further the kinetics (transport and clearance) of Aβ-lipoproteins, include liver derived lipoproteins and hepatocytes/liver involvement and contribution to plasma Aβ-lipoprotein pool. Serial ultracentrifugation can be used to isolate TRLs from IDL and HDLs at 229,190 g for 24 h (Mamo et al. 2008). Thereafter, the lipoprotein-Aβ fractions can be immunoprecipitated using monoclonal Aβ antibodies and immunoblot. Analysis of Aβ-lipoprotein fractions can be achieved by semi-dry western blotting (Mamo et al. 2008). For detection of intestinally derived CMs with Aβ, CMs can be isolated in Caco-2 cell culture or human plasma (Karpe and Hamsten 1994). This method could possibly be adapted to isolate CM fraction from VLDL and apply immunoprecipitation to quantify Aβ-CM in plasma or cell culture. The secretion of CM-Aβ can be measured in the future by collecting the conditioned medium of Caco-2 cells, then isolating the lipoprotein by sequential ultracentrifugation, followed

by SDS-PAGE and immunoblot with Aβ antibodies as described (Koudinov and Koudinova 1997). Once isolation of CM-Aβ has been established, dietary interventions with animal models, cell culture, kinetic tracer studies and clinical studies involving cognitive tests can be used to further investigate the role of CM-Aβ in AD.

The effect of apo E genotype and apo B-lipoproteins and risk in AD can be either measured with respect to the isoform of apo E – 2, 3 and 4 and the association of CM with Aβ (measure by immunoprecipitation as mentioned above) using either transgenic apo E mouse models or in clinical studies with humans with different apo E genotypes. Measurement of postprandial levels and clearance of CM-Aβ should also be studied with respect to high fat diet. The permeability of BBB, brain Aβ-lipoproteins-apo B can be quantified and correlated with plasma results where possible.

6.4 Summary

The findings presented in this thesis suggest that dietary fatty acids promote the expression of Aβ within enterocytes, possibly via greater synthesis from APP. Grant (2014) showed that in eight countries, increased consumption of animal fat (high SFA) and calories positively correlated with the increased prevalence of AD. In addition, several studies show that SFA specifically increases AD risk whereas PUFA and MUFA can reduce risk (Eskelinen et al. 2008, Kalmijn et al. 1997, Luchsinger et al. 2002, Molteni et al. 2002, Sofrizzi et al. 2005, Grant 1998, Grant 2014). However, the equal increases in enterocytic Aβ by intake of SFA, MUFA and PUFA diets could mean that modulation of AD risk by dietary fats would likely occur via pathways other than enterocytic production and secretion of Aβ. As suggested by subsequent studies from our laboratory, SFA but not MUFA or PUFA might be harmful towards BBB structure and therefore compromise the bidirectional transport of Aβ between the blood and brain, favouring influx and contributing to cerebral plaque Aβ load (Takechi et al. 2009). Results in this study would support the notion that increased availability of enterocytic Aβ via diet can add to the peripheral Aβ-lipoprotein pool which can enter the brain and over time contribute to cerebral pathology (Takechi et al. 2010a). Furthermore, caution is exercised for chronic

ingestion of fat (all types) as it may increase enterocytic Aβ abundance, increased villi height and the length of the small intestine and therefore increase overall intestinal Aβ and plasma Aβ-apo B-lipoproteins (chapter 3). Based on our findings, increasing clearance or decreasing production of apo B-lipoproteins coupled with maintenance of BBB integrity may decrease cerebral Aβ load and therefore reduce risk of developing AD.

Bateman et al. (2012) showed that cerebral amyloidosis can develop over 25 years prior to the onset of cognitive decline symptoms. This study indicates that dietary fat can exacerbate the exposure of the BBB by increasing plasma and CSF Aβ-lipoproteins. Since the disease process in AD is a steady and progressive process, one can assume that chronic intake of fat will exacerbate the development of AD. This is supported by epidemiological studies that show high fat intake, especially SFA, is detrimental to cognitive state. Results from chapter 5 indicate that the abundance of intestinal Aβ increases in response to fatty acids irrespective of chain length and saturation which leads to the question as to whether unsaturated fats is protective towards AD via other mechanisms, possibly to do with increased rate of clearance and BBB integrity. Studies in our laboratory indicate that SFA is detrimental to the structure of the BBB which can lead to increase influx of Aβ-lipoprotein into the brain.

Future research would need to long term study involve human subjects, food frequency questionnaire, measurement of plasma Aβ-lipoproteins and BBB integrity to track development of AD in response to intestinally derived Aβ. It would be interesting to elucidate if chronic increase in dietary SFA, MUFA and PUFA mid-life leads to changes in plasma Aβ-lipoprotein and BBB disturbance that may eventuate into cognitive decline.

6.5 Conclusion

Findings in this thesis are the first to explore the likelihood that small intestinal enterocytes may contribute Aβ-lipoproteins in response to increased intake of calories via dietary fat. Intake of SFA, MUFA and PUFA all increase enterocytic Aβ abundance and increase enterocyte number but SFA specifically causes

damage to BBB which indicates high intake of SFA alone can contribute over time to pathology of AD. I suspect that dietary SFA induced increases in enterocytic expression of Aβ and secretion of Aβ-apo B-lipoproteins can contribute over time and perhaps decades before the onset of cognitive symptoms to the eventual development of AD. This thesis presents a mechanistic link between two multi-factorial concepts: dietary lipid metabolism and Aβ production in a wild-type murine model.

Bibliography

Bibliography

Abad-Rodriguez J, Ledesma MD, Craessaerts K, Perga S, Medina M, Delacourte A, Dingwall C, De Strooper B & Dotti CG. (2004) Neuronal membrane cholesterol loss enhances amyloid peptide generation. *J Cell Biol.* 167, 953-960.

Abbott NJ, Patabendige AA, Dolman DE, Yusof SR & Begley DJ. (2010) Structure and function of the blood-brain barrier. *Neurobiol Dis.* 37, 13-25.

Abuznait AH, Qosa H, Busnena BA, El Sayed KA & Kaddoumi A. (2013) Olive-oil-derived oleocanthal enhances beta-amyloid clearance as a potential neuroprotective mechanism against Alzheimer's disease: in vitro and in vivo studies. *ACS Chem Neurosci.* 4, 973-982.

Adunsky A, Chesnin V, Davidson M, Gerber Y, Alexander K & Haratz D. (2002) A cross-sectional study of lipids and ApoC levels in Alzheimer's patients with and without cardiovascular disease. *J Gerontol A Biol Sci Med Sci.* 57, 757-761.

Akiyama H, Barger S, Barnum S, Bradt B, Bauer J, Cole GM, Cooper NR, Eikelenboom P, Emmerling M, Fiebich BL, Finch CE, Frautschy S, Griffin WS, Hampel H, Hull M, Landreth G, Lue L, Mrak R, Mackenzie IR, McGeer PL, O'Banion MK, Pachter J, Pasinetti G, Plata-Salaman C, Rogers J, Rydel R, Shen Y, Streit W, Strohmeyer R, Tooyoma I, Van Muiswinkel FL, Veerhuis R, Walker D, Webster S, Wegrzyniak B, Wenk G & Wyss-Coray T. (2000) Inflammation and Alzheimer's disease. *Neurobiol Aging.* 21, 383-421.

Albanese E, Dangour AD, Uauy R, Acosta D, Guerra M, Guerra SS, Huang Y, Jacob KS, de Rodriguez JL, Noriega LH, Salas A, Sosa AL, Sousa RM, Williams J, Ferri CP & Prince MJ. (2009) Dietary fish and meat intake and dementia in Latin America, China, and India: a 10/66 Dementia Research Group population-based study. *Am J Clin Nutr.* 90, 392-400.

Allsop D, Landon M & Kidd M. (1983) The isolation and amino acid composition of senile plaque core protein. *Brain Res.* 259, 348-352.

Alonso AC, Grundke-Iqbal I & Iqbal K. (1996) Alzheimer's disease hyperphosphorylated tau sequesters normal tau into tangles of filaments and disassembles microtubules. *Nat Med.* 2, 783-787.

Alsop DC, Detre JA & Grossman M. (2000) Assessment of cerebral blood flow in Alzheimer's disease by spin-labeled magnetic resonance imaging. *Ann Neurol.* 47, 93-100.

Altman R & Rutledge JC. (2010) The vascular contribution to Alzheimer's disease. *Clin Sci (Lond).* 119, 407-421.

Amtul Z, Uhrig M, Rozmahel RF & Beyreuther K. (2011a) Structural insight into the differential effects of omega-3 and omega-6 fatty acids on the production of Abeta peptides and amyloid plaques. *J Biol Chem.* 286, 6100-6107.

Amtul Z, Uhrig M, Supino R & Beyreuther K. (2010) Phospholipids and a phospholipid-rich diet alter the in vitro amyloid-beta peptide levels and amyloid-beta 42/40 ratios. *Neurosci Lett.* 481, 73-77.

Amtul Z, Westaway D, Cechetto DF & Rozmahel RF. (2011b) Oleic acid ameliorates amyloidosis in cellular and mouse models of Alzheimer's disease. *Brain Pathol.* 21, 321-329.

Anderson GJ, Tso PS & Connor WE. (1994) Incorporation of chylomicron fatty acids into the developing rat brain. *J Clin Invest.* 93, 2764-2767.

Andreasen N & Blennow K. (2002) Beta-amyloid (Abeta) protein in cerebrospinal fluid as a biomarker for Alzheimer's disease. *Peptides.* 23, 1205-1214.

Arendash GW, Su GC, Crawford FC, Bjugstad KB & Mullan M. (1999) Intravascular beta-amyloid infusion increases blood pressure: implications for a vasoactive role of beta-amyloid in the pathogenesis of Alzheimer's disease. *Neurosci Lett.* 268, 17-20.

Arendt T, Holzer M, Fruth R, Bruckner MK & Gartner U. (1998) Phosphorylation of tau, Abeta-formation, and apoptosis after in vivo inhibition of PP-1 and PP-2A. *Neurobiol Aging.* 19, 3-13.

Arrol S, Mackness MI & Durrington PN. (2000) The effects of fatty acids on apolipoprotein B secretion by human hepatoma cells (HEP G2). *Atherosclerosis.* 150, 255-264.

Arsenault D, Julien C, Tremblay C & Calon F. (2011) DHA improves cognition and prevents dysfunction of entorhinal cortex neurons in 3xTg-AD mice. *PLoS One.* 6, 17397.

Austin MA. (1999) Epidemiology of hypertriglyceridemia and cardiovascular disease. *Am J Cardiol.* 83, 13F-16F.

Australian Bureau of Statistics. (2008) Population Projections, Australia, 2006 to 2101. Cat no. 3222.0. *ABS*. Canberra.

Australian Bureau of Statistics. (2012) Australian Demographic Statistics, Jun 2012. Cat no. 3101.0. *ABS*. Canberra.

Australian Institute of Health and Welfare. (2012) Dementia in Australia. Cat no. AGE 90 AIHW, Canberra.

Balabanov R & Dore-Duffy P. (1998) Role of the CNS microvascular pericyte in the blood-brain barrier. *J Neurosci Res.* 53, 637-644.

Bales KR, Verina T, Cummins DJ, Du Y, Dodel RC, Saura J, Fishman CE, DeLong CA, Piccardo P, Petegnief V, Ghetti B & Paul SM. (1999) Apolipoprotein E is

essential for amyloid deposition in the APP(V717F) transgenic mouse model of Alzheimer's disease. *Proc Natl Acad Sci U S A.* 96, 15233-15238.

Ballabh P, Braun A & Nedergaard M. (2004) The blood-brain barrier: an overview: structure, regulation, and clinical implications. *Neurobiol Dis.* 16, 1-13.

Ballard C, Gauthier S, Corbett A, Brayne C, Aarsland D & Jones E. (2011) Alzheimer's disease. *Lancet.* 377, 1019-1031.

Bame KJ, Danda J, Hassall A & Tumova S. (1997) Abeta(1-40) prevents heparanase-catalyzed degradation of heparan sulfate glycosaminoglycans and proteoglycans in vitro. A role for heparan sulfate proteoglycan turnover in Alzheimer's disease. *J Biol Chem.* 272, 17005-17011.

Barberger-Gateau P, Letenneur L, Deschamps V, Peres K, Dartigues JF & Renaud S. (2002) Fish, meat, and risk of dementia: cohort study. *BMJ.* 325, 932-933.

Barnham KJ, McKinstry WJ, Multhaup G, Galatis D, Morton CJ, Curtain CC, Williamson NA, White AR, Hinds MG, Norton RS, Beyreuther K, Masters CL, Parker MW & Cappai R. (2003) Structure of the Alzheimer's disease amyloid precursor protein copper binding domain. A regulator of neuronal copper homeostasis. *J Biol Chem.* 278, 17401-17407.

Barrow CJ, Yasuda A, Kenny PT & Zagorski MG. (1992) Solution conformations and aggregational properties of synthetic amyloid beta-peptides of Alzheimer's disease. Analysis of circular dichroism spectra. *J Mol Biol.* 225, 1075-1093.

Bateman PA, Jackson KG, Maitin V, Yaqoob P & Williams CM. (2007) Differences in cell morphology, lipid and apo B secretory capacity in caco-2 cells following long term treatment with saturated and monounsaturated fatty acids. *Biochim Biophys Acta.* 1771, 475-485.

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

Beach TG, Wilson JR, Sue LI, Newell A, Poston M, Cisneros R, Pandya Y, Esh C, Connor DJ, Sabbagh M, Walker DG & Roher AE. (2007) Circle of Willis atherosclerosis: association with Alzheimer's disease, neuritic plaques and neurofibrillary tangles. *Acta Neuropathol.* 113, 13-21.

Bellosta S, Ferri N, Bernini F, Paoletti R & Corsini A. (2000) Non-lipid-related effects of statins. *Ann Med.* 32, 164-176.

Beyreuther K, Pollwein P, Multhaup G, Monning U, Konig G, Dyrks T, Schubert W & Masters CL. (1993) Regulation and expression of the Alzheimer's beta/A4 amyloid protein precursor in health, disease, and Down's syndrome. *Ann N Y Acad Sci.* 695, 91-102.

Biere AL, Ostaszewski B, Stimson ER, Hyman BT, Maggio JE & Selkoe DJ. (1996) Amyloid beta-peptide is transported on lipoproteins and albumin in human plasma. *J Biol Chem.* 271, 32916-32922.

Blessed G, Tomlinson BE & Roth M. (1968) The association between quantitative measures of dementia and of senile change in the cerebral grey matter of elderly subjects. *Br J Psychiatry.* 114, 797-811.

Boyt AA, Taddei K, Hallmayer J, Mamo J, Helmerhorst E, Gandy SE & Martins RN. (1999) Relationship between lipid metabolism and amyloid precursor protein and apolipoprotein E. *Alzheimer's Reports.* 2, 339-346.

Brandtzaeg P. (1998) The increasing power of immunohistochemistry and immunocytochemistry. *J Immunol Methods.* 216, 49-67.

Brantley PJ, Myers VH & Roy HJ. (2005) Environmental and lifestyle influences on obesity. *J La State Med Soc.* 157 Spec No 1, S19-27.

Breteler MM. (2000) Vascular risk factors for Alzheimer's disease: an epidemiologic perspective. *Neurobiol Aging.* 21, 153-160.

Brookmeyer R, Johnson E, Ziegler-Graham K & Arrighi HM. (2007) Forecasting the global burden of Alzheimer's disease. *Alzheimers Dement.* 3, 186-191.

Brownlow ML, Benner L, D'Agostino D, Gordon MN & Morgan D. (2013) Ketogenic diet improves motor performance but not cognition in two mouse models of Alzheimer's pathology. *PLoS One.* 8, e75713.

Burgess BL, McIsaac SA, Naus KE, Chan JY, Tansley GH, Yang J, Miao F, Ross CJ, van Eck M, Hayden MR, van Nostrand W, St George-Hyslop P, Westaway D & Wellington CL. (2006) Elevated plasma triglyceride levels precede amyloid deposition in Alzheimer's disease mouse models with abundant A beta in plasma. *Neurobiol Dis.* 24, 114-127.

Busciglio J, Gabuzda DH, Matsudaira P & Yankner BA. (1993) Generation of beta-amyloid in the secretory pathway in neuronal and nonneuronal cells. *Proc Natl Acad Sci U S A.* 90, 2092-2096.

Busciglio J, Lorenzo A, Yeh J & Yankner BA. (1995) beta-amyloid fibrils induce tau phosphorylation and loss of microtubule binding. *Neuron.* 14, 879-888.

Calon F, Lim GP, Morihara T, Yang F, Ubeda O, Salem N, Jr., Frautschy SA & Cole GM. (2005) Dietary n-3 polyunsaturated fatty acid depletion activates caspases and decreases NMDA receptors in the brain of a transgenic mouse model of Alzheimer's disease. *Eur J Neurosci.* 22, 617-626.

Calon F, Lim GP, Yang F, Morihara T, Teter B, Ubeda O, Rostaing P, Triller A, Salem N, Jr., Ashe KH, Frautschy SA & Cole GM. (2004) Docosahexaenoic acid protects from dendritic pathology in an Alzheimer's disease mouse model. *Neuron.* 43, 633-645.

Campos E, Nakajima K, Tanaka A & Havel RJ. (1992) Properties of an apolipoprotein E-enriched fraction of triglyceride-rich lipoproteins isolated from human blood plasma with a monoclonal antibody to apolipoprotein B-100. *J Lipid Res.* 33, 369-380.

Caramelli P, Nitrini R, Maranhao R, Lourenco AC, Damasceno MC, Vinagre C & Caramelli B. (1999) Increased apolipoprotein B serum concentration in Alzheimer's disease. *Acta Neurol Scand.* 100, 61-63.

Carpentier AC. (2008) Postprandial fatty acid metabolism in the development of lipotoxicity and type 2 diabetes. *Diabetes Metab.* 34, 97-107.

Carter DB, Dunn E, McKinley DD, Stratman NC, Boyle TP, Kuiper SL, Oostveen JA, Weaver RJ, Boller JA & Gurney ME. (2001) Human apolipoprotein E4 accelerates beta-amyloid deposition in APPsw transgenic mouse brain. *Ann Neurol.* 50, 468-475.

Chan JC, Tong PC & Critchley JA. (2002) The insulin resistance syndrome: mechanisms of clustering of cardiovascular risk. *Semin Vasc Med.* 2, 45-57.

Chandra V, Ganguli M, Pandav R, Johnston J, Belle S & DeKosky ST. (1998) Prevalence of Alzheimer's disease and other dementias in rural India: the Indo-US study. *Neurology.* 51, 1000-1008.

Chen P, Ratcliff G, Belle SH, Cauley JA, DeKosky ST & Ganguli M. (2001) Patterns of cognitive decline in presymptomatic Alzheimer disease: a prospective community study. *Arch Gen Psychiatry.* 58, 853-858.

Chu K, Miyazaki M, Man WC & Ntambi JM. (2006) Stearoyl-coenzyme A desaturase 1 deficiency protects against hypertriglyceridemia and increases plasma high-density lipoprotein cholesterol induced by liver X receptor activation. *Mol Cell Biol.* 26, 6786-6798.

Claassen JA & Zhang R. (2011) Cerebral autoregulation in Alzheimer's disease. *J Cereb Blood Flow Metab.* 31, 1572-1577.

Clifford PM, Zarrabi S, Siu G, Kinsler KJ, Kosciuk MC, Venkataraman V, D'Andrea MR, Dinsmore S & Nagele RG. (2007) Abeta peptides can enter the brain through a defective blood-brain barrier and bind selectively to neurons. *Brain Res.* 1142, 223-236.

Cnop M, Igoillo-Esteve M, Cunha DA, Ladriere L & Eizirik DL. (2008) An update on lipotoxic endoplasmic reticulum stress in pancreatic beta-cells. *Biochem Soc Trans.* 36, 909-915.

Cole GM, Ma QL & Frautschy SA. (2010) Dietary fatty acids and the aging brain. *Nutr Rev.* 68 Suppl 2, 102-111.

Cole SL & Vassar R. (2006) Isoprenoids and Alzheimer's disease: a complex relationship. *Neurobiol Dis.* 22, 209-222.

Conquer JA, Tierney MC, Zecevic J, Bettger WJ & Fisher RH. (2000) Fatty acid analysis of blood plasma of patients with Alzheimer's disease, other types of dementia, and cognitive impairment. *Lipids.* 35, 1305-1312.

Cook DG, Forman MS, Sung JC, Leight S, Kolson DL, Iwatsubo T, Lee VM & Doms RW. (1997) Alzheimer's A beta(1-42) is generated in the endoplasmic reticulum/intermediate compartment of NT2N cells. *Nat Med.* 3, 1021-1023.

Corder EH, Saunders AM, Risch NJ, Strittmatter WJ, Schmechel DE, Gaskell PC, Jr., Rimmler JB, Locke PA, Conneally PM, Schmader KE & et al. (1994) Protective effect of apolipoprotein E type 2 allele for late onset Alzheimer disease. *Nat Genet.* 7, 180-184.

Corder EH, Saunders AM, Strittmatter WJ, Schmechel DE, Gaskell PC, Small GW, Roses AD, Haines JL & Pericak-Vance MA. (1993) Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science.* 261, 921-923.

Crawford F, Soto C, Suo Z, Fang C, Parker T, Sawar A, Frangione B & Mullan M. (1998) Alzheimer's beta-amyloid vasoactivity: identification of a novel beta-amyloid conformational intermediate. *FEBS Lett.* 436, 445-448.

Crawford F, Suo Z, Fang C, Sawar A, Su G, Arendash G & Mullan M. (1997) The vasoactivity of A beta peptides. *Ann N Y Acad Sci.* 826, 35-46.

Cullen KM. (1997) Perivascular astrocytes within Alzheimer's disease plaques. *Neuroreport.* 8, 1961-1966.

Cunnane SC, Plourde M, Pifferi F, Begin M, Feart C & Barberger-Gateau P. (2009) Fish, docosahexaenoic acid and Alzheimer's disease. *Prog Lipid Res.* 48, 239-256.

Cunningham KM, Daly J, Horowitz M & Read NW. (1991) Gastrointestinal adaptation to diets of differing fat composition in human volunteers. *Gut.* 32, 483-486.

Dane-Stewart CA, Watts GF, Pal S, Chan D, Thompson P, Hung J & Mamo JC. (2003) Effect of atorvastatin on apolipoprotein B48 metabolism and low-density lipoprotein receptor activity in normolipidemic patients with coronary artery disease. *Metabolism.* 52, 1279-1286.

Dauncey MJ, Ingram DL, James PS & Smith MW. (1983) Modification by diet and environmental temperature of enterocyte function in piglet intestine. *J Physiol.* 341, 441-452.

Davidson NO, Kollmer ME & Glickman RM. (1986) Apolipoprotein B synthesis in rat small intestine: regulation by dietary triglyceride and biliary lipid. *J Lipid Res.* 27, 30-39.

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

Davis-Salinas J, Saporito-Irwin SM, Cotman CW & Van Nostrand WE. (1995) Amyloid beta-protein induces its own production in cultured degenerating cerebrovascular smooth muscle cells. *J Neurochem.* 65, 931-934.

Davis HR, Jr., Zhu LJ, Hoos LM, Tetzloff G, Maguire M, Liu J, Yao X, Iyer SP, Lam MH, Lund EG, Detmers PA, Graziano MP & Altmann SW. (2004) Niemann-Pick C1 Like 1 (NPC1L1) is the intestinal phytosterol and cholesterol transporter and a key modulator of whole-body cholesterol homeostasis. *J Biol Chem.* 279, 33586-33592.

Dawkins E & Small DH. (2014) Insights into the physiological function of the beta-amyloid precursor protein: beyond Alzheimer's disease. *J Neurochem.* 129, 756-769.

de Bruin TW, Brouwer CB, van Linde-Sibenius Trip M, Jansen H & Erkelens DW. (1993) Different postprandial metabolism of olive oil and soybean oil: a possible mechanism of the high-density lipoprotein conserving effect of olive oil. *Am J Clin Nutr.* 58, 477-483.

De Lorgeril M, Salen P, Martin JL, Mamelle N, Monjaud I, Touboul P & Delaye J. (1996) Effect of a mediterranean type of diet on the rate of cardiovascular complications in patients with coronary artery disease. Insights into the cardioprotective effect of certain nutriments. *J Am Coll Cardiol.* 28, 1103-1108.

de Lorgeril M, Salen P, Martin JL, Monjaud I, Delaye J & Mamelle N. (1999) Mediterranean diet, traditional risk factors, and the rate of cardiovascular complications after myocardial infarction: final report of the Lyon Diet Heart Study. *Circulation.* 99, 779-785.

Deane R, Bell RD, Sagare A & Zlokovic BV. (2009) Clearance of amyloid-beta peptide across the blood-brain barrier: implication for therapies in Alzheimer's disease. *CNS Neurol Disord Drug Targets.* 8, 16-30.

Deane R, Du Yan S, Submamaryan RK, LaRue B, Jovanovic S, Hogg E, Welch D, Manness L, Lin C, Yu J, Zhu H, Ghiso J, Frangione B, Stern A, Schmidt AM, Armstrong DL, Arnold B, Liliensiek B, Nawroth P, Hofman F, Kindy M, Stern D & Zlokovic B. (2003) RAGE mediates amyloid-beta peptide transport across the blood-brain barrier and accumulation in brain. *Nat Med.* 9, 907-913.

Deane R, Sagare A, Hamm K, Parisi M, Lane S, Finn MB, Holtzman DM & Zlokovic BV. (2008) apoE isoform-specific disruption of amyloid beta peptide clearance from mouse brain. *J Clin Invest.* 118, 4002-4013.

Deane R, Sagare A, Hamm K, Parisi M, LaRue B, Guo H, Wu Z, Holtzman DM & Zlokovic BV. (2005) IgG-assisted age-dependent clearance of Alzheimer's amyloid beta peptide by the blood-brain barrier neonatal Fc receptor. *J Neurosci.* 25, 11495-11503.

Degrace P, Caselli C & Bernard A. (1998) Long-term adaptation to high-fat diets modifies the nature and output of postprandial intestinal lymph fatty acid in rats. *J Nutr.* 128, 185-192.

DeMattos RB. (2004) Apolipoprotein E dose-dependent modulation of beta-amyloid deposition in a transgenic mouse model of Alzheimer's disease. *J Mol Neurosci.* 23, 255-262.

Demuro A, Mina E, Kayed R, Milton SC, Parker I & Glabe CG. (2005) Calcium dysregulation and membrane disruption as a ubiquitous neurotoxic mechanism of soluble amyloid oligomers. *J Biol Chem.* 280, 17294-17300.

Devore EE, Grodstein F, van Rooij FJ, Hofman A, Rosner B, Stampfer MJ, Witteman JC & Breteler MM. (2009) Dietary intake of fish and omega-3 fatty acids in relation to long-term dementia risk. *Am J Clin Nutr.* 90, 170-176.

Diakogiannaki E & Morgan NG. (2008) Differential regulation of the ER stress response by long-chain fatty acids in the pancreatic beta-cell. *Biochem Soc Trans.* 36, 959-962.

Diaz O, Berquand A, Dubois M, Di Agostino S, Sette C, Bourgoin S, Lagarde M, Nemoz G & Prigent AF. (2002) The mechanism of docosahexaenoic acid-induced phospholipase D activation in human lymphocytes involves exclusion of the enzyme from lipid rafts. *J Biol Chem.* 277, 39368-39378.

Dodart JC, Bales KR, Gannon KS, Greene SJ, DeMattos RB, Mathis C, DeLong CA, Wu S, Wu X, Holtzman DM & Paul SM. (2002) Immunization reverses memory deficits without reducing brain Abeta burden in Alzheimer's disease model. *Nat Neurosci.* 5, 452-457.

Donahue JE, Flaherty SL, Johanson CE, Duncan JA, 3rd, Silverberg GD, Miller MC, Tavares R, Yang W, Wu Q, Sabo E, Hovanesian V & Stopa EG. (2006) RAGE, LRP-1, and amyloid-beta protein in Alzheimer's disease. *Acta Neuropathol.* 112, 405-415.

Donahue JE & Johanson CE. (2008) Apolipoprotein E, amyloid-beta, and blood-brain barrier permeability in Alzheimer disease. *J Neuropathol Exp Neurol.* 67, 261-270.

Dosunmu R, Wu J, Basha MR & Zawia NH. (2007) Environmental and dietary risk factors in Alzheimer's disease. *Expert Rev Neurother.* 7, 887-900.

Driscoll DM & Getz GS. (1984) Extrahepatic synthesis of apolipoprotein E. *J Lipid Res.* 25, 1368-1379.

Dubois C, Armand M, Azais-Braesco V, Portugal H, Pauli AM, Bernard PM, Latge C, Lafont H, Borel P & Lairon D. (1994) Effects of moderate amounts of emulsified dietary fat on postprandial lipemia and lipoproteins in normolipidemic adults. *Am J Clin Nutr.* 60, 374-382.

Dulubova I, Ho A, Huryeva I, Sudhof TC & Rizo J. (2004) Three-dimensional structure of an independently folded extracellular domain of human amyloid-beta precursor protein. *Biochemistry.* 43, 9583-9588.

Eckert GP, Wood WG & Muller WE. (2005) Statins: drugs for Alzheimer's disease? *J Neural Transm.* 112, 1057-1071.

Eikelenboom P, Veerhuis R, Scheper W, Rozemuller AJ, van Gool WA & Hoozemans JJ. (2006) The significance of neuroinflammation in understanding Alzheimer's disease. *J Neural Transm.* 113, 1685-1695.

Ellis RJ, Olichney JM, Thal LJ, Mirra SS, Morris JC, Beekly D & Heyman A. (1996) Cerebral amyloid angiopathy in the brains of patients with Alzheimer's disease: the CERAD experience, Part XV. *Neurology.* 46, 1592-1596.

Elvang AB, Volbracht C, Pedersen LO, Jensen KG, Karlsson JJ, Larsen SA, Mork A, Stensbol TB & Bastlund JF. (2009) Differential effects of gamma-secretase and BACE1 inhibition on brain Abeta levels in vitro and in vivo. *J Neurochem.* 110, 1377-1387.

Emanuele E, Peros E, Tomaino C, Feudatari E, Bernardi L, Binetti G, Maletta R, D'Angelo A, Montagna L, Bruni AC & Geroldi D. (2004) Apolipoprotein(a) null phenotype is related to a delayed age at onset of Alzheimer's disease. *Neurosci Lett.* 357, 45-48.

Engelhart MJ, Geerlings MI, Ruitenberg A, Van Swieten JC, Hofman A, Witteman JC & Breteler MM. (2002) Diet and risk of dementia: Does fat matter?: The Rotterdam Study. *Neurology.* 59, 1915-1921.

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

Esler WP, Stimson ER, Ghilardi JR, Vinters HV, Lee JP, Mantyh PW & Maggio JE. (1996) In vitro growth of Alzheimer's disease beta-amyloid plaques displays first-order kinetics. *Biochemistry.* 35, 749-757.

Esteban JA. (2004) Living with the enemy: a physiological role for the beta-amyloid peptide. *Trends Neurosci.* 27, 1-3.

Farrer LA, Cupples LA, Haines JL, Hyman B, Kukull WA, Mayeux R, Myers RH, Pericak-Vance MA, Risch N & van Duijn CM. (1997) Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. APOE and Alzheimer Disease Meta Analysis Consortium. *JAMA.* 278, 1349-1356.

Farrer LA, Friedland RP, Bowirrat A, Waraska K, Korczyn A & Baldwin CT. (2003) Genetic and environmental epidemiology of Alzheimer's disease in arabs residing in Israel. *J Mol Neurosci.* 20, 207-212.

Fassbender K, Simons M, Bergmann C, Stroick M, Lutjohann D, Keller P, Runz H, Kuhl S, Bertsch T, von Bergmann K, Hennerici M, Beyreuther K & Hartmann

T. (2001) Simvastatin strongly reduces levels of Alzheimer's disease beta - amyloid peptides Abeta 42 and Abeta 40 in vitro and in vivo. *Proc Natl Acad Sci U S A.* 98, 5856-5861.

Feart C, Samieri C, Rondeau V, Amieva H, Portet F, Dartigues JF, Scarmeas N & Barberger-Gateau P. (2009) Adherence to a Mediterranean diet, cognitive decline, and risk of dementia. *JAMA.* 302, 638-648.

Feng B, Yao PM, Li Y, Devlin CM, Zhang D, Harding HP, Sweeney M, Rong JX, Kuriakose G, Fisher EA, Marks AR, Ron D & Tabas I. (2003) The endoplasmic reticulum is the site of cholesterol-induced cytotoxicity in macrophages. *Nat Cell Biol.* 5, 781-792.

Ferri CP, Prince M, Brayne C, Brodaty H, Fratiglioni L, Ganguli M, Hall K, Hasegawa K, Hendrie H, Huang Y, Jorm A, Mathers C, Menezes PR, Rimmer E, Scazufca M & Alzheimer's Disease I. (2005) Global prevalence of dementia: a Delphi consensus study. *Lancet.* 366, 2112-2117.

Field FJ, Albright E & Mathur SN. (1988) Regulation of triglyceride-rich lipoprotein secretion by fatty acids in CaCo-2 cells. *J Lipid Res.* 29, 1427-1437.

Flood C, Gustafsson M, Richardson PE, Harvey SC, Segrest JP & Boren J. (2002) Identification of the proteoglycan binding site in apolipoprotein B48. *J Biol Chem.* 277, 32228-32233.

Fotuhi M, Mohassel P & Yaffe K. (2009) Fish consumption, long-chain omega-3 fatty acids and risk of cognitive decline or Alzheimer disease: a complex association. *Nat Clin Pract Neurol.* 5, 140-152.

Frears ER, Stephens DJ, Walters CE, Davies H & Austen BM. (1999) The role of cholesterol in the biosynthesis of beta-amyloid. *Neuroreport.* 10, 1699-1705.

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

Freund-Levi Y, Eriksdotter-Jonhagen M, Cederholm T, Basun H, Faxen-Irving G, Garlind A, Vedin I, Vessby B, Wahlund LO & Palmblad J. (2006) Omega-3 fatty acid treatment in 174 patients with mild to moderate Alzheimer disease: OmegAD study: a randomized double-blind trial. *Arch Neurol.* 63, 1402-1408.

Fryer JD, Simmons K, Parsadanian M, Bales KR, Paul SM, Sullivan PM & Holtzman DM. (2005) Human apolipoprotein E4 alters the amyloid-beta 40:42 ratio and promotes the formation of cerebral amyloid angiopathy in an amyloid precursor protein transgenic model. *J Neurosci.* 25, 2803-2810.

Galloway S, Jian L, Johnsen R, Chew S & Mamo JC. (2007) beta-amyloid or its precursor protein is found in epithelial cells of the small intestine and is stimulated by high-fat feeding. *J Nutr Biochem.* 18, 279-284.

Galloway S, Pallebage-Gamarallage MM, Takechi R, Jian L, Johnsen RD, Dhaliwal SS & Mamo JC. (2008) Synergistic effects of high fat feeding and apolipoprotein E deletion on enterocytic amyloid-beta abundance. *Lipids Health Dis.* 7, 15.

Galloway S, Takechi R, Pallebage-Gamarallage MM, Dhaliwal SS & Mamo JC. (2009) Amyloid-beta colocalizes with apolipoprotein B in absorptive cells of the small intestine. *Lipids Health Dis.* 8, 46.

Gamoh S, Hashimoto M, Hossain S & Masumura S. (2001) Chronic administration of docosahexaenoic acid improves the performance of radial arm maze task in aged rats. *Clin Exp Pharmacol Physiol.* 28, 266-270.

Gardener S, Gu Y, Rainey-Smith SR, Keogh JB, Clifton PM, Mathieson SL, Taddei K, Mondal A, Ward VK, Scarmeas N, Barnes M, Ellis KA, Head R, Masters CL, Ames D, Macaulay SL, Rowe CC, Szoeke C, Martins RN & Group AR. (2012) Adherence to a Mediterranean diet and Alzheimer's disease risk in an Australian population. *Transl Psychiatry.* 2, 164.

Gearing M, Mori H & Mirra SS. (1996) Abeta-peptide length and apolipoprotein E genotype in Alzheimer's disease. *Ann Neurol.* 39, 395-399.

George AJ, Holsinger RM, McLean CA, Laughton KM, Beyreuther K, Evin G, Masters CL & Li QX. (2004) APP intracellular domain is increased and soluble Abeta is reduced with diet-induced hypercholesterolemia in a transgenic mouse model of Alzheimer disease. *Neurobiol Dis.* 16, 124-132.

Gershkovich P & Hoffman A. (2005) Uptake of lipophilic drugs by plasma derived isolated chylomicrons: linear correlation with intestinal lymphatic bioavailability. *Eur J Pharm Sci.* 26, 394-404.

Ghersi-Egea JF, Gorevic PD, Ghiso J, Frangione B, Patlak CS & Fenstermacher JD. (1996) Fate of cerebrospinal fluid-borne amyloid beta-peptide: rapid clearance into blood and appreciable accumulation by cerebral arteries. *J Neurochem.* 67, 880-883.

Ghiselli G, Schaefer EJ, Zech LA, Gregg RE & Brewer HB, Jr. (1982) Increased prevalence of apolipoprotein E4 in type V hyperlipoproteinemia. *J Clin Invest.* 70, 474-477.

Ghiso J, Gardella JE, Liem L, Gorevic PD & Frangione B. (1994) Characterization of a novel processing pathway for Alzheimer's amyloid beta precursor protein. *Neurosci Lett.* 171, 213-216.

Ghiso J, Matsubara E, Koudinov A, Choi-Miura NH, Tomita M, Wisniewski T & Frangione B. (1993) The cerebrospinal-fluid soluble form of Alzheimer's amyloid beta is complexed to SP-40,40 (apolipoprotein J), an inhibitor of the complement membrane-attack complex. *Biochem J.* 293, 27-30.

Ghribi O, Golovko MY, Larsen B, Schrag M & Murphy EJ. (2006) Deposition of iron and beta-amyloid plaques is associated with cortical cellular damage in rabbits fed with long-term cholesterol-enriched diets. *J Neurochem.* 99, 438-449.

Giubilei F, D'Antona R, Antonini R, Lenzi GL, Ricci G & Fieschi C. (1990) Serum lipoprotein pattern variations in dementia and ischemic stroke. *Acta Neurol Scand.* 81, 84-86.

Glenner GG & Wong CW. (1984) Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. *Biochem Biophys Res Commun.* 120, 885-890.

Golde TE & Eckman CB. (2001) Cholesterol modulation as an emerging strategy for the treatment of Alzheimer's disease. *Drug Discov Today.* 6, 1049-1055.

Golde TE, Estus S, Usiak M, Younkin LH & Younkin SG. (1990) Expression of beta amyloid protein precursor mRNAs: recognition of a novel alternatively spliced form and quantitation in Alzheimer's disease using PCR. *Neuron.* 4, 253-267.

Goldgaber D, Schwarzman AI, Bhasin R, Gregori L, Schmechel D, Saunders AM, Roses AD & Strittmatter WJ. (1993) Sequestration of amyloid beta-peptide. *Ann N Y Acad Sci.* 695, 139-143.

Gonzalez S, Huerta JM, Fernandez S, Patterson AM & Lasheras C. (2010) The relationship between dietary lipids and cognitive performance in an elderly population. *Int J Food Sci Nutr.* 61, 217-225.

Grant WB. (1998) The APOE-epsilon4 allele and Alzheimer disease among African Americans, Hispanics, and whites. *JAMA.* 280, 1662-1663; author reply 1663.

Grant WB (2014) Trends in diet and Alzheimer's disease during the nutrition transition in Japan and developing countries. *J Alzheimers Dis.* 38, 611-620.

Graves AB, Rajaram L, Bowen JD, McCormick WC, McCurry SM & Larson EB. (1999) Cognitive decline and Japanese culture in a cohort of older Japanese

Americans in King County, WA: the Kame Project. *J Gerontol B Psychol Sci Soc Sci.* 54, S154-161.

Green KN, Martinez-Coria H, Khashwji H, Hall EB, Yurko-Mauro KA, Ellis L & LaFerla FM. (2007) Dietary docosahexaenoic acid and docosapentaenoic acid ameliorate amyloid-beta and tau pathology via a mechanism involving presenilin 1 levels. *J Neurosci.* 27, 4385-4395.

Green PH & Riley JW. (1981) Lipid absorption and intestinal lipoprotein formation. *Aust N Z J Med.* 11, 84-90.

Greenwood CE & Winocur G. (1990) Learning and memory impairment in rats fed a high saturated fat diet. *Behav Neural Biol.* 53, 74-87.

Greenwood CE & Winocur G. (2001) Glucose treatment reduces memory deficits in young adult rats fed high-fat diets. *Neurobiol Learn Mem.* 75, 179-189.

Gregg RE, Zech LA, Schaefer EJ, Stark D, Wilson D & Brewer HB, Jr. (1986) Abnormal in vivo metabolism of apolipoprotein E4 in humans. *J Clin Invest.* 78, 815-821.

Gustafson D. (2008) A life course of adiposity and dementia. *Eur J Pharmacol.* 585, 163-175.

Haass C, Koo EH, Capell A, Teplow DB & Selkoe DJ. (1995) Polarized sorting of beta-amyloid precursor protein and its proteolytic products in MDCK cells is regulated by two independent signals. *J Cell Biol.* 128, 537-547.

Haass C, Schlossmacher MG, Hung AY, Vigo-Pelfrey C, Mellon A, Ostaszewski BL, Lieberburg I, Koo EH, Schenk D, Teplow DB & et al. (1992) Amyloid beta-peptide is produced by cultured cells during normal metabolism. *Nature.* 359, 322-325.

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

Hamelin BA & Turgeon J. (1998) Hydrophilicity/lipophilicity: relevance for the pharmacology and clinical effects of HMG-CoA reductase inhibitors. *Trends Pharmacol Sci.* 19, 26-37.

Hanson AJ, Bayer-Carter JL, Green PS, Montine TJ, Wilkinson CW, Baker LD, Watson GS, Bonner LM, Callaghan M, Leverenz JB, Tsai E, Postupna N, Zhang J, Lampe J & Craft S. (2013) Effect of apolipoprotein E genotype and diet on apolipoprotein E lipidation and amyloid peptides: randomized clinical trial. *JAMA Neurol.* 70, 972-980.

Hardy J, Duff K, Hardy KG, Perez-Tur J & Hutton M. (1998) Genetic dissection of Alzheimer's disease and related dementias: amyloid and its relationship to tau. *Nat Neurosci.* 1, 355-358.

Harper JD & Lansbury PT, Jr. (1997) Models of amyloid seeding in Alzheimer's disease and scrapie: mechanistic truths and physiological consequences of the time-dependent solubility of amyloid proteins. *Annu Rev Biochem.* 66, 385-407.

Harper JD, Wong SS, Lieber CM & Lansbury PT, Jr. (1999) Assembly of A beta amyloid protofibrils: an in vitro model for a possible early event in Alzheimer's disease. *Biochemistry.* 38, 8972-8980.

Hashimoto M, Hossain S, Agdul H & Shido O. (2005) Docosahexaenoic acid-induced amelioration on impairment of memory learning in amyloid beta-infused rats relates to the decreases of amyloid beta and cholesterol levels in detergent-insoluble membrane fractions. *Biochim Biophys Acta.* 1738, 91-98.

Hatters DM, Peters-Libeu CA & Weisgraber KH. (2006) Apolipoprotein E structure: insights into function. *Trends Biochem Sci.* 31, 445-454.

Havlik RJ, Izmirlian G, Petrovitch H, Ross GW, Masaki K, Curb JD, Saunders AM, Foley DJ, Brock D, Launer LJ & White L. (2000) APOE-epsilon4 predicts incident AD in Japanese-American men: the honolulu-asia aging study. *Neurology.* 54, 1526-1529.

Hawkins BT & Davis TP. (2005) The blood-brain barrier/neurovascular unit in health and disease. *Pharmacol Rev.* 57, 173-185.

Hayes KC, Khosla P, Hajri T & Pronczuk A. (1997) Saturated fatty acids and LDL receptor modulation in humans and monkeys. *Prostaglandins Leukot Essent Fatty Acids.* 57, 411-418.

Hedin HL, Nasman A & Fowler CJ. (1998) The secretion of soluble amyloid beta precursor protein (A beta PPs) by chick neurons in serum-free primary culture is not regulated by protein kinase C. *Amyloid.* 5, 227-237.

Heeren J, Niemeier A, Merkel M & Beisiegel U. (2002) Endothelial-derived lipoprotein lipase is bound to postprandial triglyceride-rich lipoproteins and mediates their hepatic clearance in vivo. *J Mol Med (Berl).* 80, 576-584.

Hendrie HC, Baiyewu O, Eldemire D & Prince C. (1996) Cross-cultural perspectives: Caribbean, Native American, and Yoruba. *Int Psychogeriatr.* 8 Suppl 3, 483-486.

Hendrie HC, Osuntokun BO, Hall KS, Ogunniyi AO, Hui SL, Unverzagt FW, Gureje O, Rodenberg CA, Baiyewu O & Musick BS. (1995) Prevalence of Alzheimer's disease and dementia in two communities: Nigerian Africans and African Americans. *Am J Psychiatry.* 152, 1485-1492.

Ho L, Qin W, Pompl PN, Xiang Z, Wang J, Zhao Z, Peng Y, Cambareri G, Rocher A, Mobbs CV, Hof PR & Pasinetti GM. (2004) Diet-induced insulin resistance promotes amyloidosis in a transgenic mouse model of Alzheimer's disease. *FASEB J.* 18, 902-904.

Honig LS, Tang MX, Albert S, Costa R, Luchsinger J, Manly J, Stern Y & Mayeux R. (2003) Stroke and the risk of Alzheimer disease. *Arch Neurol.* 60, 1707-1712.

Hooijmans CR, Van der Zee CE, Dederen PJ, Brouwer KM, Reijmer YD, van Groen T, Broersen LM, Lutjohann D, Heerschap A & Kiliaan AJ. (2009) DHA and cholesterol containing diets influence Alzheimer-like pathology, cognition and cerebral vasculature in APPswe/PS1dE9 mice. *Neurobiol Dis.* 33, 482-498.

Hsiao KK, Borchelt DR, Olson K, Johannsdottir R, Kitt C, Yunis W, Xu S, Eckman C, Younkin S, Price D & et al. (1995) Age-related CNS disorder and early death in transgenic FVB/N mice overexpressing Alzheimer amyloid precursor proteins. *Neuron.* 15, 1203-1218.

Hui DY, Innerarity TL & Mahley RW. (1981) Lipoprotein binding to canine hepatic membranes. Metabolically distinct apo-E and apo-B,E receptors. *J Biol Chem.* 256, 5646-5655.

Hultin M & Olivecrona T. (1998) Conversion of chylomicrons into remnants. *Atherosclerosis.* 141 Suppl 1, 25-29.

Hussain MM. (2014) Intestinal lipid absorption and lipoprotein formation. *Curr Opin Lipidol.* 25, 200-206.

Hussain MM, Kancha RK, Zhou Z, Luchoomun J, Zu H & Bakillah A. (1996) Chylomicron assembly and catabolism: role of apolipoproteins and receptors. *Biochim Biophys Acta.* 1300, 151-170.

Hussain MM, Kedees MH, Singh K, Athar H & Jamali NZ. (2001) Signposts in the assembly of chylomicrons. *Front Biosci.* 6, 320-331.

Ikeda K, Haga C & Kosaka K. (1990) Light and electron microscopic examination of amyloid-rich primitive plaques: comparison with diffuse plaques. *J Neurol.* 237, 88-93.

Iozzo RV. (1998) Matrix proteoglycans: from molecular design to cellular function. *Annu Rev Biochem.* 67, 609-652.

Irizarry MC, Deng A, Lleo A, Berezovska O, Von Arnim CA, Martin-Rehrmann M, Manelli A, LaDu MJ, Hyman BT & Rebeck GW. (2004) Apolipoprotein E modulates gamma-secretase cleavage of the amyloid precursor protein. *J Neurochem.* 90, 1132-1143.

Isherwood SG, Williams CM & Gould BJ. (1997) Apolipoprotein B-48 as a marker for chylomicrons and their remnants: studies in the postprandial state. *Proc Nutr Soc.* 56, 497-505.

Itagaki S, McGeer PL, Akiyama H, Zhu S & Selkoe D. (1989) Relationship of microglia and astrocytes to amyloid deposits of Alzheimer disease. *J Neuroimmunol.* 24, 173-182.

Iwata H, Tomita T, Maruyama K & Iwatsubo T. (2001) Subcellular compartment and molecular subdomain of beta-amyloid precursor protein relevant to the Abeta 42-promoting effects of Alzheimer mutant presenilin 2. *J Biol Chem.* 276, 21678-21685.

Iwatsubo T, Odaka A, Suzuki N, Mizusawa H, Nukina N & Ihara Y. (1994) Visualization of A beta 42(43) and A beta 40 in senile plaques with end-specific A beta monoclonals: evidence that an initially deposited species is A beta 42(43). *Neuron.* 13, 45-53.

Jackle S, Rinninger F, Greeve J, Greten H & Windler E. (1992) Regulation of the hepatic removal of chylomicron remnants and beta-very low density lipoproteins in the rat. *J Lipid Res.* 33, 419-429.

Jackson KG, Maitin V, Leake DS, Yaqoob P & Williams CM. (2006) Saturated fat-induced changes in Sf 60-400 particle composition reduces uptake of LDL by HepG2 cells. *J Lipid Res.* 47, 393-403.

Jackson KG, Robertson MD, Fielding BA, Frayn KN & Williams CM. (2002) Olive oil increases the number of triacylglycerol-rich chylomicron particles compared with other oils: an effect retained when a second standard meal is fed. *Am J Clin Nutr.* 76, 942-949.

Jackson KG, Wolstencroft EJ, Bateman PA, Yaqoob P & Williams CM. (2005) Acute effects of meal fatty acids on postprandial NEFA, glucose and apo E response: implications for insulin sensitivity and lipoprotein regulation? *Br J Nutr.* 93, 693-700.

James AP & Mamo JC. (2005) The immunodetection of lipoprotein-bound amyloid-beta is attenuated because of the presence of lipids. *Ann Clin Biochem.* 42, 70-72.

James AP, Pal S, Gennat HC, Vine DF & Mamo JC. (2003) The incorporation and metabolism of amyloid-beta into chylomicron-like lipid emulsions. *J Alzheimers Dis.* 5, 179-188.

Jancso G, Domoki F, Santha P, Varga J, Fischer J, Orosz K, Penke B, Becskei A, Dux M & Toth L. (1998) Beta-amyloid (1-42) peptide impairs blood-brain barrier function after intracarotid infusion in rats. *Neurosci Lett.* 253, 139-141.

Jiao S, Moberly JB & Schonfeld G. (1990) Editing of apolipoprotein B messenger RNA in differentiated Caco-2 cells. *J Lipid Res.* 31, 695-700.

Jick H, Zornberg GL, Jick SS, Seshadri S & Drachman DA. (2000) Statins and the risk of dementia. *Lancet.* 356, 1627-1631.

Jofre-Monseny L, Minihane AM & Rimbach G. (2008) Impact of apoE genotype on oxidative stress, inflammation and disease risk. *Mol Nutr Food Res.* 52, 131-145.

Johnson NA, Jahng GH, Weiner MW, Miller BL, Chui HC, Jagust WJ, Gorno-Tempini ML & Schuff N. (2005) Pattern of cerebral hypoperfusion in Alzheimer disease and mild cognitive impairment measured with arterial spin-labeling MR imaging: initial experience. *Radiology.* 234, 851-859.

Jonsson T, Atwal JK, Steinberg S, Snaedal J, Jonsson PV, Bjornsson S, Stefansson H, Sulem P, Gudbjartsson D, Maloney J, Hoyte K, Gustafson A, Liu Y, Lu Y, Bhangale T, Graham RR, Huttenlocher J, Bjornsdottir G, Andreassen OA, Jonsson EG, Palotie A, Behrens TW, Magnusson OT, Kong A, Thorsteinsdottir U, Watts RJ & Stefansson K. (2012) A mutation in APP protects against Alzheimer's disease and age-related cognitive decline. *Nature.* 488, 96-99.

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

Kalaria RN. (1992) The blood-brain barrier and cerebral microcirculation in Alzheimer disease. *Cerebrovasc Brain Metab Rev.* 4, 226-260.

Kalaria RN, Akinyemi R & Ihara M. (2012) Does vascular pathology contribute to Alzheimer changes? *J Neurol Sci.* 322, 141-147.

Kalback W, Esh C, Castano EM, Rahman A, Kokjohn T, Luehrs DC, Sue L, Cisneros R, Gerber F, Richardson C, Bohrmann B, Walker DG, Beach TG & Roher AE. (2004) Atherosclerosis, vascular amyloidosis and brain hypoperfusion in the pathogenesis of sporadic Alzheimer's disease. *Neurol Res.* 26, 525-539.

Kalmijn S. (2000) Fatty acid intake and the risk of dementia and cognitive decline: a review of clinical and epidemiological studies. *J Nutr Health Aging.* 4, 202-207.

Kalmijn S, Launer LJ, Ott A, Witteman JC, Hofman A & Breteler MM. (1997) Dietary fat intake and the risk of incident dementia in the Rotterdam Study. *Ann Neurol.* 42, 776-782.

Kandimalla KK, Curran GL, Holasek SS, Gilles EJ, Wengenack TM & Poduslo JF. (2005) Pharmacokinetic analysis of the blood-brain barrier transport of 125I-amyloid beta protein 40 in wild-type and Alzheimer's disease transgenic mice (APP,PS1) and its implications for amyloid plaque formation. *J Pharmacol Exp Ther.* 313, 1370-1378.

Kanemitsu N, Shimamoto C, Hiraike Y, Omae T, Iwakura K, Nakanishi Y & Katsu K. (2006) Fat Absorption and Morphological Changes in the Small Intestine in Model Mice with Hyperlipidemia (Apo E Deficiency). *Bulletin of Osaka Medical College.* 52, 59-67.

Kang J, Lemaire HG, Unterbeck A, Salbaum JM, Masters CL, Grzeschik KH, Multhaup G, Beyreuther K & Muller-Hill B. (1987) The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. *Nature.* 325, 733-736.

Kanoski SE & Davidson TL. (2011) Western diet consumption and cognitive impairment: links to hippocampal dysfunction and obesity. *Physiol Behav.* 103, 59-68.

Kaplan RJ & Greenwood CE. (1998) Dietary saturated fatty acids and brain function. *Neurochem Res.* 23, 615-626.

Kastelein JJ, van der Steeg WA, Holme I, Gaffney M, Cater NB, Barter P, Deedwania P, Olsson AG, Boekholdt SM, Demicco DA, Szarek M, LaRosa JC, Pedersen TR, Grundy SM, Group TNTS & Group IS. (2008) Lipids, apolipoproteins, and their ratios in relation to cardiovascular events with statin treatment. *Circulation.* 117, 3002-3009.

Katsiardanis K, Diamantaras AA, Dessypris N, Michelakos T, Anastasiou A, Katsiardani KP, Kanavidis P, Papadopoulos FC, Stefanadis C, Panagiotakos DB & Petridou ET. (2013) Cognitive impairment and dietary habits among elders: the Velestino Study. *J Med Food.* 16, 343-350.

Kesaniemi YA, Ehnholm C & Miettinen TA. (1987) Intestinal cholesterol absorption efficiency in man is related to apoprotein E phenotype. *J Clin Invest.* 80, 578-581.

King IS, Paterson JY, Peacock MA, Smith MW & Syme G. (1983) Effect of diet upon enterocyte differentiation in the rat jejunum. *J Physiol.* 344, 465-481.

Kivipelto M, Helkala EL, Laakso MP, Hanninen T, Hallikainen M, Alhainen K, Soininen H, Tuomilehto J & Nissinen A. (2001) Midlife vascular risk factors and Alzheimer's disease in later life: longitudinal, population based study. *BMJ.* 322, 1447-1451.

Kivipelto M, Laakso MP, Tuomilehto J, Nissinen A & Soininen H. (2002) Hypertension and hypercholesterolaemia as risk factors for Alzheimer's disease: potential for pharmacological intervention. *CNS Drugs.* 16, 435-444.

Klegeris A & McGeer PL. (2002) Cyclooxygenase and 5-lipoxygenase inhibitors protect against mononuclear phagocyte neurotoxicity. *Neurobiol Aging.* 23, 787-794.

Klein WL. (2002) Abeta toxicity in Alzheimer's disease: globular oligomers (ADDLs) as new vaccine and drug targets. *Neurochem Int.* 41, 345-352.

Kobayashi M, Ohno T, Tsuchiya T & Horio F. (2004) Characterization of diabetes-related traits in MSM and JF1 mice on high-fat diet. *J Nutr Biochem.* 15, 614-621.

Kontush A. (2004) Apolipoprotein Abeta: black sheep in a good family. *Brain Pathol.* 14, 433-447.

Koudinov A, Matsubara E, Frangione B & Ghiso J. (1994) The soluble form of Alzheimer's amyloid beta protein is complexed to high density lipoprotein 3 and very high density lipoprotein in normal human plasma. *Biochem Biophys Res Commun.* 205, 1164-1171.

Koudinov AR & Koudinova NV. (1997) Alzheimer's soluble amyloid beta protein is secreted by HepG2 cells as an apolipoprotein. *Cell Biol Int.* 21, 265-271.

Koudinov AR, Koudinova NV & Berezov TT. (1996a) Alzheimer's peptides A beta 1-40 and A beta 1-28 inhibit the plasma cholesterol esterification rate. *Biochem Mol Biol Int.* 38, 747-752.

Koudinov AR, Koudinova NV, Kumar A, Beavis RC & Ghiso J. (1996b) Biochemical characterization of Alzheimer's soluble amyloid beta protein in human cerebrospinal fluid: association with high density lipoproteins. *Biochem Biophys Res Commun.* 223, 592-597.

Koudinova NV, Berezov TT & Koudinov AR. (1996) Multiple inhibitory effects of Alzheimer's peptide Abeta1-40 on lipid biosynthesis in cultured human HepG2 cells. *FEBS Lett.* 395, 204-206.

Krapp A, Ahle S, Kersting S, Hua Y, Kneser K, Nielsen M, Gliemann J & Beisiegel U. (1996) Hepatic lipase mediates the uptake of chylomicrons and beta-VLDL into cells via the LDL receptor-related protein (LRP). *J Lipid Res.* 37, 926-936.

Kuehl FA, Jr. & Egan RW. (1980) Prostaglandins, arachidonic acid, and inflammation. *Science.* 210, 978-984.

Kuipers F, Jong MC, Lin Y, Eck M, Havinga R, Bloks V, Verkade HJ, Hofker MH, Moshage H, Berkel TJ, Vonk RJ & Havekes LM. (1997) Impaired secretion of very low density lipoprotein-triglycerides by apolipoprotein E- deficient mouse hepatocytes. *J Clin Invest.* 100, 2915-2922.

Kuo YM, Emmerling MR, Bisgaier CL, Essenburg AD, Lampert HC, Drumm D & Roher AE. (1998) Elevated low-density lipoprotein in Alzheimer's disease correlates with brain abeta 1-42 levels. *Biochem Biophys Res Commun.* 252, 711-715.

Kuo YM, Emmerling MR, Lampert HC, Hempelman SR, Kokjohn TA, Woods AS, Cotter RJ & Roher AE. (1999) High levels of circulating Abeta42 are sequestered by plasma proteins in Alzheimer's disease. *Biochem Biophys Res Commun.* 257, 787-791.

Kurokawa M, Hirano T, Furukawa S, Nagano S & Adachi M. (1995) Similar to oleic acid, eicosapentaenoic acid stimulates apolipoprotein B secretion by inhibiting its intracellular degradation in Hep G2 cells. *Atherosclerosis.* 112, 59-68.

LaDu MJ, Lukens JR, Reardon CA & Getz GS. (1997) Association of human, rat, and rabbit apolipoprotein E with beta-amyloid. *J Neurosci Res.* 49, 9-18.

LaDu MJ, Pederson TM, Frail DE, Reardon CA, Getz GS & Falduto MT. (1995) Purification of apolipoprotein E attenuates isoform-specific binding to beta-amyloid. *J Biol Chem.* 270, 9039-9042.

La Fata G, Weber P & Mohajeri MH. (2014) Effects of vitamin E on cognitive performance during ageing and in Alzheimer's disease. *Nutrients.* 6, 5453-5472.

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

Lam V, Takechi R, Pallebage-Gamarallage MM, Galloway S & Mamo JC. (2011) Colocalisation of plasma derived apo B lipoproteins with cerebral proteoglycans in a transgenic-amyloid model of Alzheimer's disease. *Neurosci Lett.* 492, 160-164.

Lange KL, Bondi MW, Salmon DP, Galasko D, Delis DC, Thomas RG & Thal LJ. (2002) Decline in verbal memory during preclinical Alzheimer's disease: examination of the effect of APOE genotype. *J Int Neuropsychol Soc.* 8, 943-955.

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

Laurin D, Verreault R, Lindsay J, Dewailly E & Holub BJ. (2003) Omega-3 fatty acids and risk of cognitive impairment and dementia. *J Alzheimers Dis.* 5, 315-322.

Lazarczyk MJ, Hof PR, Bouras C & Giannakopoulos P. (2012) Preclinical Alzheimer disease: identification of cases at risk among cognitively intact older individuals. *BMC Med.* 10, 127.

Lee DM & Singh S. (1988) Degradation of apolipoprotein B-100 in human chylomicrons. *Biochim Biophys Acta.* 960, 148-156.

Leighton JK, Joyner J, Zamarripa J, Deines M & Davis RA. (1990) Fasting decreases apolipoprotein B mRNA editing and the secretion of small molecular weight apoB by rat hepatocytes: evidence that the total amount of apoB secreted is regulated post-transcriptionally. *J Lipid Res.* 31, 1663-1668.

Lewczuk P, Kamrowski-Kruck H, Peters O, Heuser I, Jessen F, Popp J, Burger K, Hampel H, Frolich L, Wolf S, Prinz B, Jahn H, Luckhaus C, Perneczky R, Hull M, Schroder J, Kessler H, Pantel J, Gertz HJ, Klafki HW, Kolsch H, Reulbach U, Esselmann H, Maler JM, Bibl M, Kornhuber J & Wiltfang J.

(2010) Soluble amyloid precursor proteins in the cerebrospinal fluid as novel potential biomarkers of Alzheimer's disease: a multicenter study. *Mol Psychiatry.* 15, 138-145.

Lewis RA, Austen KF & Soberman RJ. (1990) Leukotrienes and other products of the 5-lipoxygenase pathway. Biochemistry and relation to pathobiology in human diseases. *N Engl J Med.* 323, 645-655.

Li M, Chen L, Lee DH, Yu LC & Zhang Y. (2007) The role of intracellular amyloid beta in Alzheimer's disease. *Prog Neurobiol.* 83, 131-139.

Lim GP, Calon F, Morihara T, Yang F, Teter B, Ubeda O, Salem N, Jr., Frautschy SA & Cole GM. (2005) A diet enriched with the omega-3 fatty acid docosahexaenoic acid reduces amyloid burden in an aged Alzheimer mouse model. *J Neurosci.* 25, 3032-3040.

Lim SY & Suzuki H. (2000) Intakes of dietary docosahexaenoic acid ethyl ester and egg phosphatidylcholine improve maze-learning ability in young and old mice. *J Nutr.* 130, 1629-1632.

Lindsay J, Laurin D, Verreault R, Hebert R, Helliwell B, Hill GB & McDowell I. (2002) Risk factors for Alzheimer's disease: a prospective analysis from the Canadian Study of Health and Aging. *Am J Epidemiol.* 156, 445-453.

Lippa CF, Nee LE, Mori H & St George-Hyslop P. (1998) Abeta-42 deposition precedes other changes in PS-1 Alzheimer's disease. *Lancet.* 352, 1117-1118.

Liu Y, Yang L, Conde-Knape K, Beher D, Shearman MS & Shachter NS. (2004) Fatty acids increase presenilin-1 levels and [gamma]-secretase activity in PSwt-1 cells. *J Lipid Res.* 45, 2368-2376.

Lorenzo A & Yankner BA. (1994) Beta-amyloid neurotoxicity requires fibril formation and is inhibited by congo red. *Proc Natl Acad Sci U S A.* 91, 12243-12247.

Luchoomun J & Hussain MM. (1999) Assembly and secretion of chylomicrons by differentiated Caco-2 cells. Nascent triglycerides and preformed phospholipids are preferentially used for lipoprotein assembly. *J Biol Chem.* 274, 19565-19572.

Luchsinger JA & Mayeux R. (2004) Cardiovascular risk factors and Alzheimer's disease. *Curr Atheroscler Rep.* 6, 261-266.

Luchsinger JA, Tang MX, Shea S & Mayeux R. (2002) Caloric intake and the risk of Alzheimer disease. *Arch Neurol.* 59, 1258-1263.

Lue LF, Kuo YM, Roher AE, Brachova L, Shen Y, Sue L, Beach T, Kurth JH, Rydel RE & Rogers J. (1999) Soluble amyloid beta peptide concentration as a predictor of synaptic change in Alzheimer's disease. *Am J Pathol.* 155, 853-862.

Lukiw WJ, Cui JG, Marcheselli VL, Bodker M, Botkjaer A, Gotlinger K, Serhan CN & Bazan NG. (2005) A role for docosahexaenoic acid-derived neuroprotectin D1 in neural cell survival and Alzheimer disease. *J Clin Invest.* 115, 2774-2783.

Mackic JB, Bading J, Ghiso J, Walker L, Wisniewski T, Frangione B & Zlokovic BV. (2002) Circulating amyloid-beta peptide crosses the blood-brain barrier in aged monkeys and contributes to Alzheimer's disease lesions. *Vascul Pharmacol.* 38, 303-313.

Mahley RW. (1988) Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. *Science.* 240, 622-630.

Mahley RW & Huang Y. (1999) Apolipoprotein E: from atherosclerosis to Alzheimer's disease and beyond. *Curr Opin Lipidol.* 10, 207-217.

Mahley RW, Hui DY, Innerarity TL & Weisgraber KH. (1981) Two independent lipoprotein receptors on hepatic membranes of dog, swine, and man. Apo-B,E and apo-E receptors. *J Clin Invest.* 68, 1197-1206.

Mahley RW & Rall SC, Jr. (2000) Apolipoprotein E: far more than a lipid transport protein. *Annu Rev Genomics Hum Genet.* 1, 507-537.

Mamo JC, Bowler A, Elsegood CL & Redgrave TG. (1991) Defective plasma clearance of chylomicron-like lipid emulsions in Watanabe heritable hyperlipidemic rabbits. *Biochim Biophys Acta.* 1081, 241-245.

Mamo JC, Elsegood CL, Umeda Y, Hirano T & Redgrave TG. (1993) Effect of probucol on plasma clearance and organ uptake of chylomicrons and VLDLs in normal and diabetic rats. *Arterioscler Thromb.* 13, 231-239.

Mamo JC, Jian L, James AP, Flicker L, Esselmann H & Wiltfang J. (2008) Plasma lipoprotein beta-amyloid in subjects with Alzheimer's disease or mild cognitive impairment. *Ann Clin Biochem.* 45, 395-403.

Mamo JC, Smith D, Yu KC, Kawaguchi A, Harada-Shiba M, Yamamura T & Yamamoto A. (1998) Accumulation of chylomicron remnants in homozygous subjects with familial hypercholesterolaemia. *Eur J Clin Invest.* 28, 379-384.

Mamo JC, Watts GF, Barrett PH, Smith D, James AP & Pal S. (2001) Postprandial dyslipidemia in men with visceral obesity: an effect of reduced LDL receptor expression? *Am J Physiol Endocrinol Metab.* 281, 626-632.

Manders EMM, Verbeek FJ & Atenm JA. (1993) Measurement of co-localization of objects in dual-colour confocal images. *J Microsc.* 169, 375-382.

Maness LM, Banks WA, Podlisny MB, Selkoe DJ & Kastin AJ. (1994) Passage of human amyloid beta-protein 1-40 across the murine blood-brain barrier. *Life Sci.* 55, 1643-1650.

Martel CL, Mackic JB, Matsubara E, Governale S, Miguel C, Miao W, McComb JG, Frangione B, Ghiso J & Zlokovic BV. (1997) Isoform-specific effects of apolipoproteins E2, E3, and E4 on cerebral capillary sequestration and blood-brain barrier transport of circulating Alzheimer's amyloid beta. *J Neurochem.* 69, 1995-2004.

Martel CL, Mackic JB, McComb JG, Ghiso J & Zlokovic BV. (1996) Blood-brain barrier uptake of the 40 and 42 amino acid sequences of circulating Alzheimer's amyloid beta in guinea pigs. *Neurosci Lett.* 206, 157-160.

Masters CL, Multhaup G, Simms G, Pottgiesser J, Martins RN & Beyreuther K. (1985) Neuronal origin of a cerebral amyloid: neurofibrillary tangles of Alzheimer's disease contain the same protein as the amyloid of plaque cores and blood vessels. *EMBO J.* 4, 2757-2763.

Matos LL, Trufelli DC, de Matos MG & da Silva Pinhal MA. (2010) Immunohistochemistry as an important tool in biomarkers detection and clinical practice. *Biomark Insights.* 5, 9-20.

Matsubara E, Ghiso J, Frangione B, Amari M, Tomidokoro Y, Ikeda Y, Harigaya Y, Okamoto K & Shoji M. (1999) Lipoprotein-free amyloidogenic peptides in plasma are elevated in patients with sporadic Alzheimer's disease and Down's syndrome. *Ann Neurol.* 45, 537-541.

Mattson MP. (2003a) Gene-diet interactions in brain aging and neurodegenerative disorders. *Ann Intern Med.* 139, 441-444.

Mattson MP. (2004) Pathways towards and away from Alzheimer's disease. *Nature.* 430, 631-639.

Maugeais C, Tietge UJ, Tsukamoto K, Glick JM & Rader DJ. (2000) Hepatic apolipoprotein E expression promotes very low density lipoprotein-apolipoprotein B production in vivo in mice. *J Lipid Res.* 41, 1673-1679.

Maurer K, Volk S & Gerbaldo H. (1997) Auguste D and Alzheimer's disease. *Lancet.* 349, 1546-1549.

Mayhan WG. (2001) Regulation of blood-brain barrier permeability. *Microcirculation.* 8, 89-104.

McGee CD & Greenwood CE. (1990) Protein and carbohydrate selection respond to changes in dietary saturated fatty acids but not to changes in essential fatty acids. *Life Sci.* 47, 67-76.

Meel-van den Abeelen AS, van Beek AH, Slump CH, Panerai RB & Claassen JA. (2014) Transfer function analysis for the assessment of cerebral autoregulation using spontaneous oscillations in blood pressure and cerebral blood flow. *Med Eng Phys.* 36, 563-575.

Menotti A, Kromhout D, Blackburn H, Fidanza F, Buzina R & Nissinen A. (1999) Food intake patterns and 25-year mortality from coronary heart disease: cross-cultural correlations in the Seven Countries Study. The Seven Countries Study Research Group. *Eur J Epidemiol.* 15, 507-515.

Mensenkamp AR, Jong MC, van Goor H, van Luyn MJ, Bloks V, Havinga R, Voshol PJ, Hofker MH, van Dijk KW, Havekes LM & Kuipers F. (1999) Apolipoprotein E participates in the regulation of very low density lipoprotein-triglyceride secretion by the liver. *J Biol Chem.* 274, 35711-35718.

Mensenkamp AR, Van Luyn MJ, Havinga R, Teusink B, Waterman IJ, Mann CJ, Elzinga BM, Verkade HJ, Zammit VA, Havekes LM, Shoulders CC & Kuipers F. (2004) The transport of triglycerides through the secretory pathway of hepatocytes is impaired in apolipoprotein E deficient mice. *J Hepatol.* 40, 599-606.

Merched A, Xia Y, Visvikis S, Serot JM & Siest G. (2000) Decreased high-density lipoprotein cholesterol and serum apolipoprotein AI concentrations are highly correlated with the severity of Alzheimer's disease. *Neurobiol Aging.* 21, 27-30.

Mikaelian I, Nanney LB, Parman KS, Kusewitt DF, Ward JM, Naf D, Krupke DM, Eppig JT, Bult CJ, Seymour R, Ichiki T & Sundberg JP. (2004) Antibodies that label paraffin-embedded mouse tissues: a collaborative endeavor. *Toxicol Pathol.* 32, 181-191.

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

Molteni R, Wu A, Vaynman S, Ying Z, Barnard RJ & Gomez-Pinilla F. (2004) Exercise reverses the harmful effects of consumption of a high-fat diet on synaptic and behavioral plasticity associated to the action of brain-derived neurotrophic factor. *Neuroscience.* 123, 429-440.

Morgan NG. (2009) Fatty acids and beta-cell toxicity. *Curr Opin Clin Nutr Metab Care.* 12, 117-122.

Morris MC. (2004) Diet and Alzheimer's disease: what the evidence shows. *MedGenMed.* 6, 48.

Morris MC, Evans DA, Bienias JL, Tangney CC, Bennett DA, Aggarwal N, Schneider J & Wilson RS. (2003) Dietary fats and the risk of incident Alzheimer disease. *Arch Neurol.* 60, 194-200.

Morris MC, Evans DA, Tangney CC, Bienias JL, Schneider JA, Wilson RS & Scherr PA. (2006) Dietary copper and high saturated and trans fat intakes associated with cognitive decline. *Arch Neurol.* 63, 1085-1088.

Nakashima Y, Plump AS, Raines EW, Breslow JL & Ross R. (1994) ApoE-deficient mice develop lesions of all phases of atherosclerosis throughout the arterial tree. *Arterioscler Thromb.* 14, 133-140.

Namba Y, Tsuchiya H & Ikeda K. (1992) Apolipoprotein B immunoreactivity in senile plaque and vascular amyloids and neurofibrillary tangles in the brains of patients with Alzheimer's disease. *Neurosci Lett.* 134, 264-266.

Naoumova RP, Dunn S, Rallidis L, Abu-Muhana O, Neuwirth C, Rendell NB, Taylor GW & Thompson GR. (1997) Prolonged inhibition of cholesterol synthesis explains the efficacy of atorvastatin. *J Lipid Res.* 38, 1496-1500.

Naqvi AZ, Harty B, Mukamal KJ, Stoddard AM, Vitolins M & Dunn JE. (2011) Monounsaturated, trans, and saturated Fatty acids and cognitive decline in women. *J Am Geriatr Soc.* 59, 837-843.

National Health and Medical Research Council, Australian Government Department of Health and Ageing, New Zealand Ministry of Health. Nutrient reference values for Australia and New Zealand including recommended dietary intakes. Canberra: Commonwealth of Australia; 2006.

Naslund J, Haroutunian V, Mohs R, Davis KL, Davies P, Greengard P & Buxbaum JD. (2000) Correlation between elevated levels of amyloid beta-peptide in the brain and cognitive decline. *JAMA.* 283, 1571-1577.

Nedergaard M, Ransom B & Goldman SA. (2003) New roles for astrocytes: redefining the functional architecture of the brain. *Trends Neurosci.* 26, 523-530.

Nelson TJ & Alkon DL. (2007) Protection against beta-amyloid-induced apoptosis by peptides interacting with beta-amyloid. *J Biol Chem.* 282, 31238-31249.

Neumann S, Coudreuse DY, van der Westhuyzen DR, Eckhardt ER, Korswagen HC, Schmitz G & Sprong H. (2009) Mammalian Wnt3a is released on lipoprotein particles. *Traffic.* 10, 334-343.

Newman EA. (2003) New roles for astrocytes: regulation of synaptic transmission. *Trends Neurosci.* 26, 536-542.

Nichols MR, Moss MA, Reed DK, Lin WL, Mukhopadhyay R, Hoh JH & Rosenberry TL. (2002) Growth of beta-amyloid(1-40) protofibrils by monomer elongation and lateral association. Characterization of distinct products by light scattering and atomic force microscopy. *Biochemistry.* 41, 6115-6127.

Nishitomi K, Sakaguchi G, Horikoshi Y, Gray AJ, Maeda M, Hirata-Fukae C, Becker AG, Hosono M, Sakaguchi I, Minami SS, Nakajima Y, Li HF, Takeyama C, Kihara T, Ota A, Wong PC, Aisen PS, Kato A, Kinoshita N & Matsuoka Y. (2006) BACE1 inhibition reduces endogenous Abeta and alters APP processing in wild-type mice. *J Neurochem.* 99, 1555-1563.

Nishitsuji K, Hosono T, Nakamura T, Bu G & Michikawa M. (2011) Apolipoprotein E regulates the integrity of tight junctions in an isoform-dependent manner in an in vitro blood-brain barrier model. *J Biol Chem.* 286, 17536-17542.

Noutsou M & Georgopoulos A. (1999) Effects of simvastatin on fasting and postprandial triglyceride-rich lipoproteins in patients with type I diabetes mellitus. *J Diabetes Complications.* 13, 98-104.

Ntambi JM & Miyazaki M. (2004) Regulation of stearoyl-CoA desaturases and role in metabolism. *Prog Lipid Res.* 43, 91-104.

O'Brien KD, Olin KL, Alpers CE, Chiu W, Ferguson M, Hudkins K, Wight TN & Chait A. (1998) Comparison of apolipoprotein and proteoglycan deposits in human coronary atherosclerotic plaques: colocalization of biglycan with apolipoproteins. *Circulation.* 98, 519-527.

Oba S, Nagata C, Nakamura K, Fujii K, Kawachi T, Takatsuka N & Shimizu H. (2009) Diet based on the Japanese Food Guide Spinning Top and subsequent mortality among men and women in a general Japanese population. *J Am Diet Assoc.* 109, 1540-1547.

Ogunniyi A & Osuntokun B. (1991) Relatively low prevalence of Alzheimer's disease in developing countries and the racial factor in dementia research. *Ethn Dis.* 1, 394-395.

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

Olin KL, Potter-Perigo S, Barrett PH, Wight TN & Chait A. (2001) Biglycan, a vascular proteoglycan, binds differently to HDL2 and HDL3: role of apoE. *Arterioscler Thromb Vasc Biol.* 21, 129-135.

Olofsson SO & Boren J. (2005) Apolipoprotein B: a clinically important apolipoprotein which assembles atherogenic lipoproteins and promotes the development of atherosclerosis. *J Intern Med.* 258, 395-410.

Olsson A, Hoglund K, Sjogren M, Andreasen N, Minthon L, Lannfelt L, Buerger K, Moller HJ, Hampel H, Davidsson P & Blennow K. (2003) Measurement of alpha- and beta-secretase cleaved amyloid precursor protein in cerebrospinal fluid from Alzheimer patients. *Exp Neurol.* 183, 74-80.

Osman I, Gaillard O, Meillet D, Bordas-Fonfrede M, Gervais A, Schuller E, Delattre J & Legrand A. (1995) A sensitive time-resolved immunofluorometric assay for the measurement of apolipoprotein B in cerebrospinal fluid. Application to multiple sclerosis and other neurological diseases. *Eur J Clin Chem Clin Biochem.* 33, 53-58.

Osuntokun BO, Ogunniyi AO & Lekwauwa UG. (1992) Alzheimer's disease in Nigeria. *Afr J Med Med Sci.* 21, 71-77.

Otsuka M, Yamaguchi K & Ueki A. (2002) Similarities and differences between Alzheimer's disease and vascular dementia from the viewpoint of nutrition. *Ann N Y Acad Sci.* 977, 155-161.

Ott A, Stolk RP, van Harskamp F, Pols HA, Hofman A & Breteler MM. (1999) Diabetes mellitus and the risk of dementia: The Rotterdam Study. *Neurology.* 53, 1937-1942.

Ott BR, Daiello LA, Dahabreh IJ, Springate BA, Bixby K, Murali M & Trikalinos TA. (2015) Do statins impair cognition? A systematic review and meta-analysis of randomized controlled trials. *J Gen Intern Med.* 30, 348-358.

Pageot LP, Perreault N, Basora N, Francoeur C, Magny P & Beaulieu JF. (2000) Human cell models to study small intestinal functions: recapitulation of the crypt-villus axis. *Microsc Res Tech.* 49, 394-406.

Pal S, Semorine K, Watts GF & Mamo J. (2003) Identification of lipoproteins of intestinal origin in human atherosclerotic plaque. *Clin Chem Lab Med.* 41, 792-795.

Pallebage-Gamarallage MM, Galloway S, Johnsen R, Jian L, Dhaliwal S & Mamo JC. (2009) The effect of exogenous cholesterol and lipid-modulating agents on enterocytic amyloid-beta abundance. *Br J Nutr.* 101, 340-347.

Pallebage-Gamarallage MM, Galloway S, Takechi R, Dhaliwal S & Mamo JC. (2012) Probucol suppresses enterocytic accumulation of amyloid-beta induced by saturated fat and cholesterol feeding. *Lipids.* 47, 27-34.

Pallebage-Gamarallage MM, Takechi R, Lam V, Galloway S, Dhaliwal S & Mamo JC. (2010) Post-prandial lipid metabolism, lipid-modulating agents and cerebrovascular integrity: implications for dementia risk. *Atheroscler Suppl.* 11, 49-54.

Panza F, D'Introno A, Colacicco AM, Basile AM, Capurso C, Kehoe PG, Capurso A & Solfrizzi V. (2004) Vascular risk and genetics of sporadic late-onset Alzheimer's disease. *J Neural Transm.* 111, 69-89.

Parhofer KG, Barrett PH & Schwandt P. (2000) Atorvastatin improves postprandial lipoprotein metabolism in normolipidemlic subjects. *J Clin Endocrinol Metab.* 85, 4224-4230.

Park IH, Hwang EM, Hong HS, Boo JH, Oh SS, Lee J, Jung MW, Bang OY, Kim SU & Mook-Jung I. (2003) Lovastatin enhances Abeta production and senile plaque deposition in female Tg2576 mice. *Neurobiol Aging.* 24, 637-643.

Parsons RB, Farrant JK, Price GC, Subramaniam D & Austen BM. (2007) Regulation of the lipidation of beta-secretase by statins. *Biochem Soc Trans.* 35, 577-582.

Patil S, Sheng L, Masserang A & Chan C. (2006) Palmitic acid-treated astrocytes induce BACE1 upregulation and accumulation of C-terminal fragment of APP in primary cortical neurons. *Neurosci Lett.* 406, 55-59.

Patterson E, Wall R, Fitzgerald GF, Ross RP & Stanton C. (2012) Health implications of high dietary omega-6 polyunsaturated Fatty acids. *J Nutr Metab.* 2012, 539426.

Pawlosky RJ, Hibbeln JR, Novotny JA & Salem N, Jr. (2001) Physiological compartmental analysis of alpha-linolenic acid metabolism in adult humans. *J Lipid Res.* 42, 1257-1265.

Peila R, Rodriguez BL, Launer LJ & Honolulu-Asia Aging S. (2002) Type 2 diabetes, APOE gene, and the risk for dementia and related pathologies: The Honolulu-Asia Aging Study. *Diabetes.* 51, 1256-1262.

Perry EK, Tomlinson BE, Blessed G, Bergmann K, Gibson PH & Perry RH. (1978) Correlation of cholinergic abnormalities with senile plaques and mental test scores in senile dementia. *Br Med J.* 2, 1457-1459.

Perry RJ & Hodges JR. (1999) Attention and executive deficits in Alzheimer's disease. A critical review. *Brain.* 122, 383-404.

Petanceska SS, Seeger M, Checler F & Gandy S. (2000) Mutant presenilin 1 increases the levels of Alzheimer amyloid beta-peptide Abeta42 in late compartments of the constitutive secretory pathway. *J Neurochem.* 74, 1878-1884.

Petot GJ & Friedland RP. (2004) Lipids, diet and Alzheimer disease: an extended summary. *J Neurol Sci.* 226, 31-33.

Pettersen JA, Sathiyamoorthy G, Gao FQ, Szilagyi G, Nadkarni NK, St George-Hyslop P, Rogaeva E & Black SE. (2008) Microbleed topography, leukoaraiosis, and cognition in probable Alzheimer disease from the Sunnybrook dementia study. *Arch Neurol.* 65, 790-795.

Pike CJ, Burdick D, Walencewicz AJ, Glabe CG & Cotman CW. (1993) Neurodegeneration induced by beta-amyloid peptides in vitro: the role of peptide assembly state. *J Neurosci.* 13, 1676-1687.

Plump AS, Smith JD, Hayek T, Aalto-Setala K, Walsh A, Verstuyft JG, Rubin EM & Breslow JL. (1992) Severe hypercholesterolemia and atherosclerosis in apolipoprotein E-deficient mice created by homologous recombination in ES cells. *Cell.* 71, 343-353.

Poduslo JF, Curran GL, Sanyal B & Selkoe DJ. (1999) Receptor-mediated transport of human amyloid beta-protein 1-40 and 1-42 at the blood-brain barrier. *Neurobiol Dis.* 6, 190-199.

Poduslo JF, Curran GL, Wengenack TM, Malester B & Duff K. (2001) Permeability of proteins at the blood-brain barrier in the normal adult mouse and double transgenic mouse model of Alzheimer's disease. *Neurobiol Dis.* 8, 555-567.

Poirier J. (2003) Apolipoprotein E and cholesterol metabolism in the pathogenesis and treatment of Alzheimer's disease. *Trends Mol Med.* 9, 94-101.

Poirier J. (2005) Apolipoprotein E, cholesterol transport and synthesis in sporadic Alzheimer's disease. *Neurobiol Aging.* 26, 355-361.

Poirier J, Davignon J, Bouthillier D, Kogan S, Bertrand P & Gauthier S. (1993) Apolipoprotein E polymorphism and Alzheimer's disease. *Lancet.* 342, 697-699.

Poirier J, Delisle MC, Quirion R, Aubert I, Farlow M, Lahiri D, Hui S, Bertrand P, Nalbantoglu J, Gilfix BM & Gauthier S. (1995) Apolipoprotein E4 allele as a predictor of cholinergic deficits and treatment outcome in Alzheimer disease. *Proc Natl Acad Sci U S A.* 92, 12260-12264.

Presecki P, Muck-Seler D, Mimica N, Pivac N, Mustapic M, Stipcevic T & Smalc VF. (2011) Serum lipid levels in patients with Alzheimer's disease. *Coll Antropol.* 35 Suppl 1, 115-120.

Prince M, Graham N, Brodaty H, Rimmer E, Varghese M, Chiu H, Acosta D & Scazufca M. (2004) Alzheimer Disease International's 10/66 Dementia Research Group - one model for action research in developing countries. *Int J Geriatr Psychiatry.* 19, 178-181.

Proctor SD & Mamo JC. (2003) Intimal retention of cholesterol derived from apolipoprotein B100- and apolipoprotein B48-containing lipoproteins in carotid arteries of Watanabe heritable hyperlipidemic rabbits. *Arterioscler Thromb Vasc Biol.* 23, 1595-1600.

Proctor SD, Vine DF & Mamo JC. (2002) Arterial retention of apolipoprotein B(48)- and B(100)-containing lipoproteins in atherogenesis. *Curr Opin Lipidol.* 13, 461-470.

Puskas LG, Kitajka K, Nyakas C, Barcelo-Coblijn G & Farkas T. (2003) Short-term administration of omega 3 fatty acids from fish oil results in increased transthyretin transcription in old rat hippocampus. *Proc Natl Acad Sci U S A.* 100, 1580-1585.

Quarfordt SH, Oswald B, Landis B, Xu HS, Zhang SH & Maeda N. (1995) In vivo cholesterol kinetics in apolipoprotein E-deficient and control mice. *J Lipid Res.* 36, 1227-1235.

Rapoport M, Dawson HN, Binder LI, Vitek MP & Ferreira A. (2002) Tau is essential to beta -amyloid-induced neurotoxicity. *Proc Natl Acad Sci U S A.* 99, 6364-6369.

Refolo LM & Fillit HM. (2004) Apolipoprotein E4 as a target for developing new therapeutics for Alzheimer's disease. *J Mol Neurosci.* 23, 151-155.

Refolo LM, Malester B, LaFrancois J, Bryant-Thomas T, Wang R, Tint GS, Sambamurti K, Duff K & Pappolla MA. (2000) Hypercholesterolemia accelerates the Alzheimer's amyloid pathology in a transgenic mouse model. *Neurobiol Dis.* 7, 321-331.

Riserus U, Arnlov J & Berglund L. (2007) Long-term predictors of insulin resistance: role of lifestyle and metabolic factors in middle-aged men. *Diabetes Care.* 30, 2928-2933.

Risau W, Esser S & Engelhardt B. (1998) Differentiation of blood-brain barrier endothelial cells. *Pathol Biol (Paris).* 46, 171-175.

Reitz C, Tang MX, Luchsinger J & Mayeux R. (2004) Relation of plasma lipids to Alzheimer disease and vascular dementia. *Arch Neurol.* 61, 705-714.

Roberts CK, Barnard RJ, Liang KH & Vaziri ND. (2002) Effect of diet on adipose tissue and skeletal muscle VLDL receptor and LPL: implications for obesity and hyperlipidemia. *Atherosclerosis.* 161, 133-141.

Roher AE, Esh C, Kokjohn TA, Kalback W, Luehrs DC, Seward JD, Sue LI & Beach TG. (2003) Circle of willis atherosclerosis is a risk factor for sporadic Alzheimer's disease. *Arterioscler Thromb Vasc Biol.* 23, 2055-2062.

Ronti T, Lupattelli G & Mannarino E. (2006) The endocrine function of adipose tissue: an update. *Clin Endocrinol (Oxf).* 64, 355-365.

Ross MH, Kanye GI & Pawlina W. (2003) Digestive system: II Esophagus and gastrointestinal tract. *Histology: a text and atlas*. Philadelphia: Lippincott Williams and Wilkins.Ruitenberg A, den Heijer T, Bakker SL, van Swieten JC, Koudstaal PJ, Hofman A & Breteler MM. (2005) Cerebral hypoperfusion and clinical onset of dementia: the Rotterdam Study. *Ann Neurol.* 57, 789-794.

Runz H, Rietdorf J, Tomic I, de Bernard M, Beyreuther K, Pepperkok R & Hartmann T. (2002) Inhibition of intracellular cholesterol transport alters presenilin localization and amyloid precursor protein processing in neuronal cells. *J Neurosci.* 22, 1679-1689.

Sabbagh M, Zahiri HR, Ceimo J, Cooper K, Gaul W, Connor D & Sparks DL. (2004) Is there a characteristic lipid profile in Alzheimer's disease? *J Alzheimers Dis.* 6, 585-589; discussion 673-581.

Sagare AP, Winkler EA, Bell RD, Deane R & Zlokovic BV. (2011) From the liver to the blood-brain barrier: an interconnected system regulating brain amyloid-beta levels. *J Neurosci Res.* 89, 967-968.

Saito H, Dhanasekaran P, Baldwin F, Weisgraber KH, Phillips MC & Lund-Katz S. (2003) Effects of polymorphism on the lipid interaction of human apolipoprotein E. *J Biol Chem.* 278, 40723-40729.

Salminen A, Ojala J, Kauppinen A, Kaarniranta K & Suuronen T. (2009) Inflammation in Alzheimer's disease: amyloid-beta oligomers trigger innate immunity defence via pattern recognition receptors. *Prog Neurobiol.* 87, 181-194.

Sanchez-Mejia RO, Newman JW, Toh S, Yu GQ, Zhou Y, Halabisky B, Cisse M, Scearce-Levie K, Cheng IH, Gan L, Palop JJ, Bonventre JV & Mucke L. (2008) Phospholipase A2 reduction ameliorates cognitive deficits in a mouse model of Alzheimer's disease. *Nat Neurosci.* 11, 1311-1318.

Sartorius T, Ketterer C, Kullmann S, Balzer M, Rotermund C, Binder S, Hallschmid M, Machann J, Schick F, Somoza V, Preissl H, Fritsche A, Haring HU & Hennige AM. (2012) Monounsaturated fatty acids prevent the aversive effects of obesity on locomotion, brain activity, and sleep behavior. *Diabetes.* 61, 1669-1679.

Saunders AM, Strittmatter WJ, Schmechel D, George-Hyslop PH, Pericak-Vance MA, Joo SH, Rosi BL, Gusella JF, Crapper-MacLachlan DR, Alberts MJ & et al. (1993) Association of apolipoprotein E allele epsilon 4 with late-onset familial and sporadic Alzheimer's disease. *Neurology.* 43, 1467-1472.

Scarmeas N, Luchsinger JA, Mayeux R & Stern Y. (2007) Mediterranean diet and Alzheimer disease mortality. *Neurology.* 69, 1084-1093.

Scarmeas N, Stern Y, Mayeux R, Manly JJ, Schupf N & Luchsinger JA. (2009) Mediterranean diet and mild cognitive impairment. *Arch Neurol.* 66, 216-225.

Schaefer EJ, Bongard V, Beiser AS, Lamon-Fava S, Robins SJ, Au R, Tucker KL, Kyle DJ, Wilson PW & Wolf PA. (2006) Plasma phosphatidylcholine docosahexaenoic acid content and risk of dementia and Alzheimer disease: the Framingham Heart Study. *Arch Neurol.* 63, 1545-1550.

Schmidt ML, DiDario AG, Otvos L, Jr., Hoshi N, Kant JA, Lee VM & Trojanowski JQ. (1994) Plaque-associated neuronal proteins: a recurrent motif in neuritic amyloid deposits throughout diverse cortical areas of the Alzheimer's disease brain. *Exp Neurol.* 130, 311-322.

Selkoe DJ. (1989) Aging, amyloid, and Alzheimer's disease. *N Engl J Med.* 320, 1484-1487.

Selkoe DJ. (2001) Alzheimer's disease results from the cerebral accumulation and cytotoxicity of amyloid beta-protein. *J Alzheimers Dis.* 3, 75-80.

Selkoe DJ, Yamazaki T, Citron M, Podlisny MB, Koo EH, Teplow DB & Haass C. (1996) The role of APP processing and trafficking pathways in the formation of amyloid beta-protein. *Ann N Y Acad Sci.* 777, 57-64.

Seubert P, Vigo-Pelfrey C, Esch F, Lee M, Dovey H, Davis D, Sinha S, Schlossmacher M, Whaley J, Swindlehurst C & et al. (1992) Isolation and quantification of soluble Alzheimer's beta-peptide from biological fluids. *Nature.* 359, 325-327.

Shadlen MF, Larson EB & Yukawa M. (2000) The epidemiology of Alzheimer's disease and vascular dementia in Japanese and African-American populations: the search for etiological clues. *Neurobiol Aging.* 21, 171-181.

Shankar GM, Li S, Mehta TH, Garcia-Munoz A, Shepardson NE, Smith I, Brett FM, Farrell MA, Rowan MJ, Lemere CA, Regan CM, Walsh DM, Sabatini BL & Selkoe DJ. (2008) Amyloid-beta protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. *Nat Med.* 14, 837-842.

Shao H, Jao S, Ma K & Zagorski MG. (1999) Solution structures of micelle-bound amyloid beta-(1-40) and beta-(1-42) peptides of Alzheimer's disease. *J Mol Biol.* 285, 755-773.

Shen H, Howles P & Tso P. (2001) From interaction of lipidic vehicles with intestinal epithelial cell membranes to the formation and secretion of chylomicrons. *Adv Drug Deliv Rev.* 50 Suppl 1, 103-125.

Sherrington R, Rogaev EI, Liang Y, Rogaeva EA, Levesque G, Ikeda M, Chi H, Lin C, Li G, Holman K, Tsuda T, Mar L, Foncin JF, Bruni AC, Montesi MP, Sorbi S, Rainero I, Pinessi L, Nee L, Chumakov I, Pollen D, Brookes A, Sanseau P, Polinsky RJ, Wasco W, Da Silva HA, Haines JL, Perkicak-Vance MA, Tanzi RE, Roses AD, Fraser PE, Rommens JM & St George-Hyslop PH. (1995) Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. *Nature.* 375, 754-760.

Shi SR, Chaiwun B, Young L, Cote RJ & Taylor CR. (1993) Antigen retrieval technique utilizing citrate buffer or urea solution for immunohistochemical

demonstration of androgen receptor in formalin-fixed paraffin sections. *J Histochem Cytochem.* 41, 1599-1604.

Shi SR, Key ME & Kalra KL. (1991) Antigen retrieval in formalin-fixed, paraffin-embedded tissues: an enhancement method for immunohistochemical staining based on microwave oven heating of tissue sections. *J Histochem Cytochem.* 39, 741-748.Shibata M, Yamada S, Kumar SR, Calero M, Bading J, Frangione B, Holtzman DM, Miller CA, Strickland DK, Ghiso J & Zlokovic BV. (2000) Clearance of Alzheimer's amyloid-ss(1-40) peptide from brain by LDL receptor-related protein-1 at the blood-brain barrier. *J Clin Invest.* 106, 1489-1499.

Shie FS, Jin LW, Cook DG, Leverenz JB & LeBoeuf RC. (2002) Diet-induced hypercholesterolemia enhances brain A beta accumulation in transgenic mice. *Neuroreport.* 13, 455-459.

Shioi J, Pangalos MN, Ripellino JA, Vassilacopoulou D, Mytilineou C, Margolis RU & Robakis NK. (1995) The Alzheimer amyloid precursor proteoglycan (appican) is present in brain and is produced by astrocytes but not by neurons in primary neural cultures. *J Biol Chem.* 270, 11839-11844.

Shoji M, Golde TE, Ghiso J, Cheung TT, Estus S, Shaffer LM, Cai XD, McKay DM, Tintner R, Frangione B & et al. (1992) Production of the Alzheimer amyloid beta protein by normal proteolytic processing. *Science.* 258, 126-129.

Simons K & Ikonen E. (2000) How cells handle cholesterol. *Science.* 290, 1721-1726.

Singh RB, Dubnov G, Niaz MA, Ghosh S, Singh R, Rastogi SS, Manor O, Pella D & Berry EM. (2002) Effect of an Indo-Mediterranean diet on progression of coronary artery disease in high risk patients (Indo-Mediterranean Diet Heart Study): a randomised single-blind trial. *Lancet.* 360, 1455-1461.

Sinha S, Anderson JP, Barbour R, Basi GS, Caccavello R, Davis D, Doan M, Dovey HF, Frigon N, Hong J, Jacobson-Croak K, Jewett N, Keim P, Knops J, Lieberburg I, Power M, Tan H, Tatsuno G, Tung J, Schenk D, Seubert P, Suomensaari SM, Wang S, Walker D, Zhao J, McConlogue L & John V. (1999) Purification and cloning of amyloid precursor protein beta-secretase from human brain. *Nature.* 402, 537-540.

Small BJ, Fratiglioni L, Viitanen M, Winblad B & Backman L. (2000) The course of cognitive impairment in preclinical Alzheimer disease: three- and 6-year follow-up of a population-based sample. *Arch Neurol.* 57, 839-844.

Small DH, Mok SS, Williamson TG & Nurcombe V. (1996) Role of proteoglycans in neural development, regeneration, and the aging brain. *J Neurochem.* 67, 889-899.

Smith D, Watts GF, Dane-Stewart C & Mamo JC. (1999) Post-prandial chylomicron response may be predicted by a single measurement of plasma apolipoprotein B48 in the fasting state. *Eur J Clin Invest.* 29, 204-209.

Snyder EM, Nong Y, Almeida CG, Paul S, Moran T, Choi EY, Nairn AC, Salter MW, Lombroso PJ, Gouras GK & Greengard P. (2005) Regulation of NMDA receptor trafficking by amyloid-beta. *Nat Neurosci.* 8, 1051-1058.

Solfrizzi V, D'Introno A, Colacicco AM, Capurso C, Del Parigi A, Capurso S, Gadaleta A, Capurso A & Panza F. (2005) Dietary fatty acids intake: possible role in cognitive decline and dementia. *Exp Gerontol.* 40, 257-270.

Solfrizzi V, Frisardi V, Capurso C, D'Introno A, Colacicco AM, Vendemiale G, Capurso A & Panza F. (2010) Dietary fatty acids in dementia and predementia syndromes: epidemiological evidence and possible underlying mechanisms. *Ageing Res Rev.* 9, 184-199.

Solfrizzi V, Panza F, Torres F, Mastroianni F, Del Parigi A, Venezia A & Capurso A. (1999) High monounsaturated fatty acids intake protects against age-related cognitive decline. *Neurology.* 52, 1563-1569.

Sparks DL. (1996) Intraneuronal beta-amyloid immunoreactivity in the CNS. *Neurobiol Aging.* 17, 291-299.

Sparks DL, Scheff SW, Hunsaker JC, 3rd, Liu H, Landers T & Gross DR. (1994) Induction of Alzheimer-like beta-amyloid immunoreactivity in the brains of rabbits with dietary cholesterol. *Exp Neurol.* 126, 88-94.

Sparks DL, Scheff SW, Liu H, Landers T, Danner F, Coyne CM & Hunsaker JC, 3rd. (1996) Increased density of senile plaques (SP), but not neurofibrillary tangles (NFT), in non-demented individuals with the apolipoprotein E4 allele: comparison to confirmed Alzheimer's disease patients. *J Neurol Sci.* 138, 97-104.

St George-Hyslop PH. (2000) Molecular genetics of Alzheimer's disease. *Biol Psychiatry.* 47, 183-199.

Strachan MW. (2003) Insulin and cognitive function. *Lancet.* 362, 1253.

Stafstrom CE & Rho JM. (2012) The ketogenic diet as a treatment paradigm for diverse neurological disorders. *Front Pharmacol.* 3, 59.

St-Onge MP, Bosarge A, Goree LL & Darnell B. (2008) Medium chain triglyceride oil consumption as part of a weight loss diet does not lead to an adverse metabolic profile when compared to olive oil. *J Am Coll Nutr.* 27, 547-552.

Strassnig M & Ganguli M. (2005) About a peculiar disease of the cerebral cortex: Alzheimer's original case revisited. *Psychiatry (Edgmont).* 2, 30-33.

Stratman NC, Castle CK, Taylor BM, Epps DE, Melchior GW & Carter DB. (2005) Isoform-specific interactions of human apolipoprotein E to an intermediate conformation of human Alzheimer amyloid-beta peptide. *Chem Phys Lipids.* 137, 52-61.

Strittmatter WJ & Roses AD. (1996) Apolipoprotein E and Alzheimer's disease. *Annu Rev Neurosci.* 19, 53-77.

Strittmatter WJ, Saunders AM, Schmechel D, Pericak-Vance M, Enghild J, Salvesen GS & Roses AD. (1993a) Apolipoprotein E: high-avidity binding to beta-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. *Proc Natl Acad Sci U S A.* 90, 1977-1981.

Strittmatter WJ, Weisgraber KH, Huang DY, Dong LM, Salvesen GS, Pericak-Vance M, Schmechel D, Saunders AM, Goldgaber D & Roses AD. (1993b) Binding of human apolipoprotein E to synthetic amyloid beta peptide: isoform-specific effects and implications for late-onset Alzheimer disease. *Proc Natl Acad Sci U S A.* 90, 8098-8102.

Studzinski CM, Li F, Bruce-Keller AJ, Fernandez-Kim SO, Zhang L, Weidner AM, Markesbery WR, Murphy MP & Keller JN. (2009) Effects of short-term Western diet on cerebral oxidative stress and diabetes related factors in APP x PS1 knock-in mice. *J Neurochem.* 108, 860-866.

Su GC, Arendash GW, Kalaria RN, Bjugstad KB & Mullan M. (1999) Intravascular infusions of soluble beta-amyloid compromise the blood-brain barrier, activate CNS glial cells and induce peripheral hemorrhage. *Brain Res.* 818, 105-117.

Subasinghe S, Unabia S, Barrow CJ, Mok SS, Aguilar MI & Small DH. (2003) Cholesterol is necessary both for the toxic effect of Abeta peptides on vascular smooth muscle cells and for Abeta binding to vascular smooth muscle cell membranes. *J Neurochem.* 84, 471-479.

Suo Z, Humphrey J, Kundtz A, Sethi F, Placzek A, Crawford F & Mullan M. (1998) Soluble Alzheimers beta-amyloid constricts the cerebral vasculature in vivo. *Neurosci Lett.* 257, 77-80.

Suryadevara V, Storey SG, Aronow WS & Ahn C. (2003) Association of abnormal serum lipids in elderly persons with atherosclerotic vascular disease and dementia, atherosclerotic vascular disease without dementia, dementia without atherosclerotic vascular disease, and no dementia or atherosclerotic vascular disease. *J Gerontol A Biol Sci Med Sci.* 58, 859-861.

Sutcliffe JG, Hedlund PB, Thomas EA, Bloom FE & Hilbush BS. (2011) Peripheral reduction of beta-amyloid is sufficient to reduce brain beta-amyloid: implications for Alzheimer's disease. *J Neurosci Res.* 89, 808-814.

Tabaton M, Nunzi MG, Xue R, Usiak M, Autilio-Gambetti L & Gambetti P. (1994) Soluble amyloid beta-protein is a marker of Alzheimer amyloid in brain but not in cerebrospinal fluid. *Biochem Biophys Res Commun.* 200, 1598-1603.

Takamura A, Kawarabayashi T, Yokoseki T, Shibata M, Morishima-Kawashima M, Saito Y, Murayama S, Ihara Y, Abe K, Shoji M, Michikawa M & Matsubara E. (2011) Dissociation of beta-amyloid from lipoprotein in cerebrospinal fluid from Alzheimer's disease accelerates beta-amyloid-42 assembly. *J Neurosci Res.* 89, 815-821.

Takechi R, Galloway S, Pallebage-Gamarallage MM & Mamo JC. (2008a) Chylomicron amyloid-beta in the aetiology of Alzheimer's disease. *Atheroscler Suppl.* 9, 19-25.

Takechi R, Galloway S, Pallebage-Gamarallage MM, Johnsen RD & Mamo JC. (2008b) Three-dimensional immunofluorescent double labelling using polyclonal antibodies derived from the same species: enterocytic colocalization of chylomicrons with Golgi apparatus. *Histochem Cell Biol.* 129, 779-784.

Takechi R, Galloway S, Pallebage-Gamarallage M, Wellington C, Johnsen R & Mamo JC. (2009) Three-dimensional colocalization analysis of plasma-derived apolipoprotein B with amyloid plaques in APP/PS1 transgenic mice. *Histochem Cell Biol.* 131, 661-666.

Takechi R, Galloway S, Pallebage-Gamarallage MM, Lam V & Mamo JC. (2010a) Dietary fats, cerebrovasculature integrity and Alzheimer's disease risk. *Prog Lipid Res.* 49, 159-170.

Takechi R, Galloway S, Pallebage-Gamarallage MM, Wellington CL, Johnsen RD, Dhaliwal SS & Mamo JC. (2010b) Differential effects of dietary fatty acids on the cerebral distribution of plasma-derived apo B lipoproteins with amyloid-beta. *Br J Nutr.* 103, 652-662.

Tangney CC, Kwasny MJ, Li H, Wilson RS, Evans DA & Morris MC. (2011) Adherence to a Mediterranean-type dietary pattern and cognitive decline in a community population. *Am J Clin Nutr.* 93, 601-607.

Tanzi RE & Bertram L. (2005) Twenty years of the Alzheimer's disease amyloid hypothesis: a genetic perspective. *Cell.* 120, 545-555.

Teng B, Burant CF & Davidson NO. (1993) Molecular cloning of an apolipoprotein B messenger RNA editing protein. *Science.* 260, 1816-1819.

Teplow DB. (1998) Structural and kinetic features of amyloid beta-protein fibrillogenesis. *Amyloid.* 5, 121-142.

Thal DR, Griffin WS, de Vos RA & Ghebremedhin E. (2008) Cerebral amyloid angiopathy and its relationship to Alzheimer's disease. *Acta Neuropathol.* 115, 599-609.

Thomas T, McLendon C, Sutton ET & Thomas G. (1997) Cerebrovascular endothelial dysfunction mediated by beta-amyloid. *Neuroreport.* 8, 1387-1391.

Thomas T, Thomas G, McLendon C, Sutton T & Mullan M. (1996) beta-Amyloid-mediated vasoactivity and vascular endothelial damage. *Nature.* 380, 168-171.

Tokuda T, Calero M, Matsubara E, Vidal R, Kumar A, Permanne B, Zlokovic B, Smith JD, Ladu MJ, Rostagno A, Frangione B & Ghiso J. (2000) Lipidation of apolipoprotein E influences its isoform-specific interaction with Alzheimer's amyloid beta peptides. *Biochem J.* 348 Pt 2, 359-365.

Tomita T, Tokuhiro S, Hashimoto T, Aiba K, Saido TC, Maruyama K & Iwatsubo T. (1998) Molecular dissection of domains in mutant presenilin 2 that mediate overproduction of amyloidogenic forms of amyloid beta peptides. Inability of truncated forms of PS2 with familial Alzheimer's disease mutation to increase secretion of Abeta42. *J Biol Chem.* 273, 21153-21160.

Treusch S, Cyr DM & Lindquist S. (2009) Amyloid deposits: protection against toxic protein species? *Cell Cycle.* 8, 1668-1674.

Tschanz JT, Norton MC, Zandi PP & Lyketsos CG. (2013) The Cache County Study on Memory in Aging: factors affecting risk of Alzheimer's disease and its progression after onset. *Int Rev Psychiatry.* 25, 673-685.

Uryu S, Tokuhiro S & Oda T. (2003) beta-Amyloid-specific upregulation of stearoyl coenzyme A desaturase-1 in macrophages. *Biochem Biophys Res Commun.* 303, 302-305.

Utermann G, Kindermann I, Kaffarnik H & Steinmetz A. (1984) Apolipoprotein E phenotypes and hyperlipidemia. *Hum Genet.* 65, 232-236.

van Gelder BM, Tijhuis M, Kalmijn S & Kromhout D. (2007) Fish consumption, n-3 fatty acids, and subsequent 5-y cognitive decline in elderly men: the Zutphen Elderly Study. *Am J Clin Nutr.* 85, 1142-1147.

van Greevenbroek MM & de Bruin TW. (1998) Chylomicron synthesis by intestinal cells in vitro and in vivo. *Atherosclerosis.* 141 Suppl 1, 9-16.

van Greevenbroek MM, van Meer G, Erkelens DW & de Bruin TW. (1996) Effects of saturated, mono-, and polyunsaturated fatty acids on the secretion of apo B containing lipoproteins by Caco-2 cells. *Atherosclerosis.* 121, 139-150.

van Horssen J, Wesseling P, van den Heuvel LP, de Waal RM & Verbeek MM. (2003) Heparan sulphate proteoglycans in Alzheimer's disease and amyloid-related disorders. *Lancet Neurol.* 2, 482-492.

van Oijen M, de Jong FJ, Witteman JC, Hofman A, Koudstaal PJ & Breteler MM. (2007) Atherosclerosis and risk for dementia. *Ann Neurol.* 61, 403-410.

Vermeer SE, Prins ND, den Heijer T, Hofman A, Koudstaal PJ & Breteler MM. (2003) Silent brain infarcts and the risk of dementia and cognitive decline. *N Engl J Med.* 348, 1215-1222.

Vigna GB, Donega P, Passaro A, Zanca R, Cattin L, Fonda M, Pauciullo P, Marotta G, Fellin R, Gasparrini S & Piliego T. (1999) Post-prandial effects of gemfibrozil vs simvastatin in hypercholesterolemic subjects with borderline hypertriglyceridemia. *Nutr Metab Cardiovasc Dis.* 9, 234-243.

Vigo-Pelfrey C, Lee D, Keim P, Lieberburg I & Schenk DB. (1993) Characterization of beta-amyloid peptide from human cerebrospinal fluid. *J Neurochem.* 61, 1965-1968.

Vincent-Baudry S, Defoort C, Gerber M, Bernard MC, Verger P, Helal O, Portugal H, Planells R, Grolier P, Amiot-Carlin MJ, Vague P & Lairon D. (2005) The Medi-RIVAGE study: reduction of cardiovascular disease risk factors after a 3-mo intervention with a Mediterranean-type diet or a low-fat diet. *Am J Clin Nutr.* 82, 964-971.

Walker RA. (2006) Quantification of immunohistochemistry--issues concerning methods, utility and semiquantitative assessment I. *Histopathology.* 49, 406-410.

Walsh DM, Klyubin I, Fadeeva JV, Cullen WK, Anwyl R, Wolfe MS, Rowan MJ & Selkoe DJ. (2002) Naturally secreted oligomers of amyloid beta protein

potently inhibit hippocampal long-term potentiation in vivo. *Nature.* 416, 535-539.

Walsh DM & Selkoe DJ. (2007) A beta oligomers - a decade of discovery. *J Neurochem.* 101, 1172-1184.

Wang J, Dickson DW, Trojanowski JQ & Lee VM. (1999) The levels of soluble versus insoluble brain Abeta distinguish Alzheimer's disease from normal and pathologic aging. *Exp Neurol.* 158, 328-337.

Wassall SR, Brzustowicz MR, Shaikh SR, Cherezov V, Caffrey M & Stillwell W. (2004) Order from disorder, corralling cholesterol with chaotic lipids. The role of polyunsaturated lipids in membrane raft formation. *Chem Phys Lipids.* 132, 79-88.

Wassall SR & Stillwell W. (2009) Polyunsaturated fatty acid-cholesterol interactions: domain formation in membranes. *Biochim Biophys Acta.* 1788, 24-32.

Weisgraber KH. (1994) Apolipoprotein E: structure-function relationships. *Adv Protein Chem.* 45, 249-302.

Weller RO, Massey A, Newman TA, Hutchings M, Kuo YM & Roher AE. (1998) Cerebral amyloid angiopathy: amyloid beta accumulates in putative interstitial fluid drainage pathways in Alzheimer's disease. *Am J Pathol.* 153, 725-733.

Wild-Bode C, Yamazaki T, Capell A, Leimer U, Steiner H, Ihara Y & Haass C. (1997) Intracellular generation and accumulation of amyloid beta-peptide terminating at amino acid 42. *J Biol Chem.* 272, 16085-16088.

Williams CM. (1997) Postprandial lipid metabolism: effects of dietary fatty acids. *Proc Nutr Soc.* 56, 679-692.

Williams CM, Bateman PA, Jackson KG & Yaqoob P. (2004) Dietary fatty acids and chylomicron synthesis and secretion. *Biochem Soc Trans.* 32, 55-58.

Wilson PW, Schaefer EJ, Larson MG & Ordovas JM. (1996) Apolipoprotein E alleles and risk of coronary disease. A meta-analysis. *Arterioscler Thromb Vasc Biol.* 16, 1250-1255.

Wimo A & Prince M. 2010. World Alzheimer's Report 2010: The Global Economic Impact of Dementia. *Alzheimer's Disease International*. London.

Winocur G & Greenwood CE. (1999) The effects of high fat diets and environmental influences on cognitive performance in rats. *Behav Brain Res.* 101, 153-161.

Wisniewski HM, Vorbrodt AW & Wegiel J. (1997a) Amyloid angiopathy and blood-brain barrier changes in Alzheimer's disease. *Ann N Y Acad Sci.* 826, 161-172.

Wisniewski HM & Wen GY. (1985) Substructures of paired helical filaments from Alzheimer's disease neurofibrillary tangles. *Acta Neuropathol.* 66, 173-176.

Wisniewski T, Ghiso J & Frangione B. (1994) Alzheimer's disease and soluble A beta. *Neurobiol Aging.* 15, 143-152.

Wisniewski T, Ghiso J & Frangione B. (1997b) Biology of A beta amyloid in Alzheimer's disease. *Neurobiol Dis.* 4, 313-328.

Wisniewski T & Sadowski M. (2008) Preventing beta-amyloid fibrillization and deposition: beta-sheet breakers and pathological chaperone inhibitors. *BMC Neurosci.* 9 Suppl 2, 5.

Wolozin B. (2004) Cholesterol, statins and dementia. *Curr Opin Lipidol.* 15, 667-672.

Wolozin B, Kellman W, Ruosseau P, Celesia GG & Siegel G. (2000) Decreased prevalence of Alzheimer disease associated with 3-hydroxy-3-methyglutaryl coenzyme A reductase inhibitors. *Arch Neurol.* 57, 1439-1443.

Woods SC, D'Alessio DA, Tso P, Rushing PA, Clegg DJ, Benoit SC, Gotoh K, Liu M & Seeley RJ. (2004) Consumption of a high-fat diet alters the homeostatic regulation of energy balance. *Physiol Behav.* 83, 573-578.

Woudstra TD, Drozdowski LA, Wild GE, Clandinin MT, Agellon LB & Thomson AB. (2004) An isocaloric PUFA diet enhances lipid uptake and weight gain in aging rats. *Lipids.* 39, 343-354.

World Health Organization. (2008) The global burden of disease: 2004 update. *WHO*. Geneva.

Wu A, Molteni R, Ying Z & Gomez-Pinilla F. (2003) A saturated-fat diet aggravates the outcome of traumatic brain injury on hippocampal plasticity and cognitive function by reducing brain-derived neurotrophic factor. *Neuroscience.* 119, 365-375.

Yamamoto K, Shimada H, Koh H, Ataka S & Miki T. (2014) Serum levels of albumin-amyloid beta complexes are decreased in Alzheimer's disease. *Geriatr Gerontol Int.* 14, 716-723.

Yan SD, Chen X, Fu J, Chen M, Zhu H, Roher A, Slattery T, Zhao L, Nagashima M, Morser J, Migheli A, Nawroth P, Stern D & Schmidt AM. (1996) RAGE and amyloid-beta peptide neurotoxicity in Alzheimer's disease. *Nature.* 382, 685-691.

Yao PM & Tabas I. (2001) Free cholesterol loading of macrophages is associated with widespread mitochondrial dysfunction and activation of the mitochondrial apoptosis pathway. *J Biol Chem.* 276, 42468-42476.

Yasojima K, McGeer EG & McGeer PL. (2001) Relationship between beta amyloid peptide generating molecules and neprilysin in Alzheimer disease and normal brain. *Brain Res.* 919, 115-121.

Ye S, Huang Y, Mullendorff K, Dong L, Giedt G, Meng EC, Cohen FE, Kuntz ID, Weisgraber KH & Mahley RW. (2005) Apolipoprotein (apo) E4 enhances amyloid beta peptide production in cultured neuronal cells: apoE structure as a potential therapeutic target. *Proc Natl Acad Sci U S A.* 102, 18700-18705.

Younkin SG. (1995) Evidence that A beta 42 is the real culprit in Alzheimer's disease. *Ann Neurol.* 37, 287-288.

Yurko-Mauro K. (2010) Cognitive and cardiovascular benefits of docosahexaenoic acid in aging and cognitive decline. *Curr Alzheimer Res.* 7, 190-196.

Yusuf S, Hawken S, Ounpuu S, Dans T, Avezum A, Lanas F, McQueen M, Budaj A, Pais P, Varigos J, Lisheng L & Investigators IS. (2004) Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. *Lancet.* 364, 937-952.

Zampelas A, Murphy M, Morgan LM & Williams CM. (1994) Postprandial lipoprotein lipase, insulin and gastric inhibitory polypeptide responses to test meals of different fatty acid composition: comparison of saturated, n-6 and n-3 polyunsaturated fatty acids. *Eur J Clin Nutr.* 48, 849-858.

Zetterberg H, Andreasson U, Hansson O, Wu G, Sankaranarayanan S, Andersson ME, Buchhave P, Londos E, Umek RM, Minthon L, Simon AJ & Blennow K. (2008) Elevated cerebrospinal fluid BACE1 activity in incipient Alzheimer disease. *Arch Neurol.* 65, 1102-1107.

Zhang SH, Reddick RL, Piedrahita JA & Maeda N. (1992) Spontaneous hypercholesterolemia and arterial lesions in mice lacking apolipoprotein E. *Science.* 258, 468-471.

Zhao Y, McCabe JB, Vance J & Berthiaume LG. (2000) Palmitoylation of apolipoprotein B is required for proper intracellular sorting and transport of cholesteroyl esters and triglycerides. *Mol Biol Cell.* 11, 721-734.

Zlokovic BV. (2005) Neurovascular mechanisms of Alzheimer's neurodegeneration. *Trends Neurosci.* 28, 202-208.

Zlokovic BV, Ghiso J, Mackic JB, McComb JG, Weiss MH & Frangione B. (1993) Blood-brain barrier transport of circulating Alzheimer's amyloid beta. *Biochem Biophys Res Commun.* 197, 1034-1040.

* Every reasonable effort has been made to acknowledge the owners of copyright material. I would be pleased to hear from any copyright holder who has been omitted or incorrectly acknowledged.

Appendices

Appendix A: Statements of contribution by Authors and copyright authorisation

This thesis consists of both original text and several research articles previously published in internationally recognised scientific journals. This thesis consists of 3 first authored articles and 5 second authored articles. All articles used in this thesis relate directly to the subject of this thesis.

Appendix A presents a statement of contribution towards each journal article as well as signature verification for these statements are provided by respective authors. Where necessary, copyright was obtained for re-production of journal articles used in and authorisation statements from respective copyright owners are provided.

First-author manuscripts

Article 1:

Galloway S, Jian L, Johnsen RD, Chew S, Mamo JCL. (2007) Beta-amyloid or its precursor protein is found in epithelial cells of the small intestine and is stimulated by high fat feeding. *J Nutr Biochem*. 18, 279-284

Susan Galloway was responsible for experimental procedures, data analysis, generation of images, and writing of the manuscript. Le Jian contributed towards experiment design, tissue collection and interpretation of results. Russel D. Johnsen contributed towards design of methodology. Stewart Chew assisted in study design and manuscript appraisal. John C. Mamo contributed to study design, interpretation of data and presentation and review of manuscript.

ELSEVIER LICENSE TERMS AND CONDITIONS

Sep 23, 2014

This is a License Agreement between Susan Galloway ("You") and Elsevier ("Elsevier") provided by Copyright Clearance Center ("CCC"). The license consists of your order details, the terms and conditions provided by Elsevier, and the payment terms and conditions.

All payments must be made in full to CCC. For payment instructions, please see information listed at the bottom of this form.

Supplier	Elsevier Limited The Boulevard,Langford Lane Kidlington,Oxford,OX5 1GB,UK
Registered Company Number	1982084
Customer name	Susan Galloway
Customer address	159 Elderberry Dr, Perth, None 6164
License number	3461310991178
License date	Sep 03, 2014
Licensed content publisher	Elsevier
Licensed content publication	The Journal of Nutritional Biochemistry
Licensed content title	β-Amyloid or its precursor protein is found in epithelial cells of the small intestine and is stimulated by high-fat feeding
Licensed content author	Susan Galloway,Le Jian,Russell Johnsen,Stewart Chew,John C.L. Mamo
Licensed content date	April 2007
Licensed content volume number	18
Licensed content issue number	4
Number of pages	6
Start Page	279
End Page	284
Type of Use	reuse in a thesis/dissertation
Portion	full article
Format	both print and electronic
Are you the author of this Elsevier article?	Yes
Will you be translating?	No
Title of your thesis/dissertation	Enterocytic beta amyloid-lipoprotein homeostasis: Implications for Alzheimer's disease risk
Expected completion date	Oct 2014
Estimated size (number of pages)	220
Elsevier VAT number	GB 494 6272 12
Price	0.00 USD
VAT/Local Sales Tax	0.00 USD / 0.00 GBP
Total	0.00 USD
Terms and Conditions	

INTRODUCTION

1. The publisher for this copyrighted material is Elsevier. By clicking "accept" in connection with completing this licensing transaction, you agree that the following terms and conditions apply to this transaction (along with the Billing and Payment terms and conditions established by Copyright Clearance Center, Inc. ("CCC"), at the time that you opened your Rightslink account and that are available at any time at http://myaccount.copyright.com).

GENERAL TERMS

2. Elsevier hereby grants you permission to reproduce the aforementioned material subject to the terms and conditions indicated.

3. Acknowledgement: If any part of the material to be used (for example, figures) has appeared in our publication with credit or acknowledgement to another source, permission must also be sought from that source. If such permission is not obtained then that material may not be included in your publication/copies. Suitable acknowledgement to the source must be made, either as a footnote or in a reference list at the end of your publication, as follows:

"Reprinted from Publication title, Vol /edition number, Author(s), Title of article / title of chapter, Pages No., Copyright (Year), with permission from Elsevier [OR APPLICABLE SOCIETY COPYRIGHT OWNER]." Also Lancet special credit - "Reprinted from The Lancet, Vol. number, Author(s), Title of article, Pages No., Copyright (Year), with permission from Elsevier."

4. Reproduction of this material is confined to the purpose and/or media for which permission is hereby given.

5. Altering/Modifying Material: Not Permitted. However figures and illustrations may be altered/adapted minimally to serve your work. Any other abbreviations, additions, deletions and/or any other alterations shall be made only with prior written authorization of Elsevier Ltd. (Please contact Elsevier at permissions@elsevier.com)

6. If the permission fee for the requested use of our material is waived in this instance, please be advised that your future requests for Elsevier materials may attract a fee.

7. Reservation of Rights: Publisher reserves all rights not specifically granted in the combination of (i) the license details provided by you and accepted in the course of this licensing transaction, (ii) these terms and conditions and (iii) CCC's Billing and Payment terms and conditions.

8. License Contingent Upon Payment: While you may exercise the rights licensed immediately upon issuance of the license at the end of the licensing process for the transaction, provided that you have disclosed complete and accurate details of your proposed use, no license is finally effective unless and until full payment is received from you (either by publisher or by CCC) as provided in CCC's Billing and Payment terms and conditions. If full payment is not received on a timely basis, then any license preliminarily granted shall be deemed automatically revoked and shall be void as if never granted. Further, in the event that you breach any of these terms and conditions or any of CCC's Billing and Payment terms and conditions, the license is automatically revoked and shall be void as if never granted. Use of materials as described in a revoked license, as well as any use of the materials beyond the scope of an unrevoked license, may constitute copyright infringement and publisher reserves the right to take any and all action to protect its copyright in the materials.

9. Warranties: Publisher makes no representations or warranties with respect to the licensed material.

10. Indemnity: You hereby indemnify and agree to hold harmless publisher and CCC, and their respective officers, directors, employees and agents, from and against any and all claims arising out of your use of the licensed material other than as specifically authorized pursuant to this license.

11. No Transfer of License: This license is personal to you and may not be sublicensed, assigned, or

transferred by you to any other person without publisher's written permission.

12. No Amendment Except in Writing: This license may not be amended except in a writing signed by both parties (or, in the case of publisher, by CCC on publisher's behalf).

13. Objection to Contrary Terms: Publisher hereby objects to any terms contained in any purchase order, acknowledgment, check endorsement or other writing prepared by you, which terms are inconsistent with these terms and conditions or CCC's Billing and Payment terms and conditions. These terms and conditions, together with CCC's Billing and Payment terms and conditions (which are incorporated herein), comprise the entire agreement between you and publisher (and CCC) concerning this licensing transaction. In the event of any conflict between your obligations established by these terms and conditions and those established by CCC's Billing and Payment terms and conditions, these terms and conditions shall control.

14. Revocation: Elsevier or Copyright Clearance Center may deny the permissions described in this License at their sole discretion, for any reason or no reason, with a full refund payable to you. Notice of such denial will be made using the contact information provided by you. Failure to receive such notice will not alter or invalidate the denial. In no event will Elsevier or Copyright Clearance Center be responsible or liable for any costs, expenses or damage incurred by you as a result of a denial of your permission request, other than a refund of the amount(s) paid by you to Elsevier and/or Copyright Clearance Center for denied permissions.

LIMITED LICENSE

The following terms and conditions apply only to specific license types:

15. **Translation**: This permission is granted for non-exclusive world **English** rights only unless your license was granted for translation rights. If you licensed translation rights you may only translate this content into the languages you requested. A professional translator must perform all translations and reproduce the content word for word preserving the integrity of the article. If this license is to re-use 1 or 2 figures then permission is granted for non-exclusive world rights in all languages.

16. **Posting licensed content on any Website**: The following terms and conditions apply as follows: Licensing material from an Elsevier journal: All content posted to the web site must maintain the copyright information line on the bottom of each image; A hyper-text must be included to the Homepage of the journal from which you are licensing at http://www.sciencedirect.com/science/journal/xxxxx or the Elsevier homepage for books at http://www.elsevier.com; Central Storage: This license does not include permission for a scanned version of the material to be stored in a central repository such as that provided by Heron/XanEdu.

Licensing material from an Elsevier book: A hyper-text link must be included to the Elsevier homepage at http://www.elsevier.com . All content posted to the web site must maintain the copyright information line on the bottom of each image.

Posting licensed content on Electronic reserve: In addition to the above the following clauses are applicable: The web site must be password-protected and made available only to bona fide students registered on a relevant course. This permission is granted for 1 year only. You may obtain a new license for future website posting.

For journal authors: the following clauses are applicable in addition to the above: Permission

granted is limited to the author accepted manuscript version* of your paper.

***Accepted Author Manuscript (AAM) Definition:** An accepted author manuscript (AAM) is the author's version of the manuscript of an article that has been accepted for publication and which may include any author-incorporated changes suggested through the processes of submission processing, peer review, and editor-author communications. AAMs do not include other publisher value-added contributions such as copy-editing, formatting, technical enhancements and (if relevant) pagination.

You are not allowed to download and post the published journal article (whether PDF or HTML, proof or final version), nor may you scan the printed edition to create an electronic version. A hyper-text must be included to the Homepage of the journal from which you are licensing at http://www.sciencedirect.com/science/journal/xxxxx. As part of our normal production process, you will receive an e-mail notice when your article appears on Elsevier's online service ScienceDirect (www.sciencedirect.com). That e-mail will include the article's Digital Object Identifier (DOI). This number provides the electronic link to the published article and should be included in the posting of your personal version. We ask that you wait until you receive this e-mail and have the DOI to do any posting.

Posting to a repository: Authors may post their AAM immediately to their employer's institutional repository for internal use only and may make their manuscript publically available after the journal-specific embargo period has ended.

Please also refer to Elsevier's Article Posting Policy for further information.

18. **For book authors** the following clauses are applicable in addition to the above: Authors are permitted to place a brief summary of their work online only.. You are not allowed to download and post the published electronic version of your chapter, nor may you scan the printed edition to create an electronic version. **Posting to a repository:** Authors are permitted to post a summary of their chapter only in their institution's repository.

20. **Thesis/Dissertation**: If your license is for use in a thesis/dissertation your thesis may be submitted to your institution in either print or electronic form. Should your thesis be published commercially, please reapply for permission. These requirements include permission for the Library and Archives of Canada to supply single copies, on demand, of the complete thesis and include permission for UMI to supply single copies, on demand, of the complete thesis. Should your thesis be published commercially, please reapply for permission.

Elsevier Open Access Terms and Conditions

Elsevier publishes Open Access articles in both its Open Access journals and via its Open Access articles option in subscription journals.

Authors publishing in an Open Access journal or who choose to make their article Open Access in an Elsevier subscription journal select one of the following Creative Commons user licenses, which define how a reader may reuse their work: Creative Commons Attribution License (CC BY), Creative Commons Attribution – Non Commercial - ShareAlike (CC BY NC SA) and Creative Commons Attribution – Non Commercial – No Derivatives (CC BY NC ND)

Terms & Conditions applicable to all Elsevier Open Access articles:

Any reuse of the article must not represent the author as endorsing the adaptation of the article nor should the article be modified in such a way as to damage the author's honour or reputation.

The author(s) must be appropriately credited.

If any part of the material to be used (for example, figures) has appeared in our publication with credit or acknowledgement to another source it is the responsibility of the user to ensure their reuse complies with the terms and conditions determined by the rights holder.

Additional Terms & Conditions applicable to each Creative Commons user license:

CC BY: You may distribute and copy the article, create extracts, abstracts, and other revised versions, adaptations or derivative works of or from an article (such as a translation), to include in a collective work (such as an anthology), to text or data mine the article, including for commercial purposes without permission from Elsevier

CC BY NC SA: For non-commercial purposes you may distribute and copy the article, create extracts, abstracts and other revised versions, adaptations or derivative works of or from an article (such as a translation), to include in a collective work (such as an anthology), to text and data mine the article and license new adaptations or creations under identical terms without permission from Elsevier

CC BY NC ND: For non-commercial purposes you may distribute and copy the article and include it in a collective work (such as an anthology), provided you do not alter or modify the article, without permission from Elsevier

Any commercial reuse of Open Access articles published with a CC BY NC SA or CC BY NC ND license requires permission from Elsevier and will be subject to a fee.

Commercial reuse includes:

· Promotional purposes (advertising or marketing)

· Commercial exploitation (e.g. a product for sale or loan)

· Systematic distribution (for a fee or free of charge)

Please refer to Elsevier's Open Access Policy for further information.

21. **Other Conditions**:

v1.6

Questions? customercare@copyright.com or +1-855-239-3415 (toll free in the US) or +1-978-646-2777.

Gratis licenses (referencing $0 in the Total field) are free. Please retain this printable license for your reference. No payment is required.

Article 2:

Galloway S, Pallebage-Gamarallage MM, Takechi R, Jian L, Johnsen RD, Dhaliwal SS, Mamo JC. (2008) Synergistic effects of high fat feeding and apolipoprotein E deletion on enterocytic amyloid beta peptide abundance*. Lipids Health Dis*. 7, 15

Susan Galloway carried out experiments, animal maintenance, collection, analysis and interpretation of data, and writing of the manuscript. Menuka M. Pallebage-Gamarallage and Ryusuke Takechi assisted in data collection and analysis. Le Jian and Satvinder S. Dhaliwal provided advice regarding statistical analysis of data. Russell D. Johnsen provided advice and assisted in method development. John C, Mamo contributed to study design, interpretation of data and critical appraisal of the manuscript.

.

Article 3:

Galloway S, Takechi R, Pallebage-Gamarallage MM, Dhaliwal SS, Mamo JC. (2009) Amyloid-beta colocalizes with apolipoprotein B in absorptive cells of the small intestine. *Lipids Health Dis.* 8, 46.

Susan Galloway was responsible for study design, collection of samples, experimental protocols and writing of the manuscript. Ryusuke Takechi contributed to development of methodology and data interpretation. Ryusuke Takechi and Menuka M. Pallebage-Gamarallage were both involved in data collection and appraisal of the draft manuscript. Satvinder S. Dhaliwal gave advice on data interpretation and statistical analysis. John C. Mamo was involved in study design, data analysis and appraisal of the draft manuscript.

Second-authored (supplementary) manuscripts

Article 1:

Takechi R, **Galloway S**, Pallebage-Gamarallage MM, Wellington C, Mamo JC. (2008) Chylomicron amyloid-beta in the aetiology of Alzheimer's disease. *Atheroscler Suppl.* 9, 19-25

Ryusuke Takechi was responsible for the review of literature, preparation and collection of data, presentation of figures and drafting of the review article. Susan Galloway and Menuka M. Pallebage-Gamarallage assisted in the presentation of data and figures and the review of manuscript. Cheryl L. Wellington supplied specimen samples for analysis and was involved in the appraisal of the manuscript. John C. Mamo was involved in the appraisal of data and review of the manuscript.

listed at the bottom of this form.

Supplier	Elsevier Limited The Boulevard,Langford Lane Kidlington,Oxford,OX5 1GB,UK
Registered Company Number	1982084
Customer name	Susan Galloway
Customer address	159 Elderberry Dr, Perth, None 6164
License number	3461960877190
License date	Sep 04, 2014
Licensed content publisher	Elsevier
Licensed content publication	Atherosclerosis Supplements
Licensed content title	Chylomicron amyloid-beta in the aetiology of Alzheimer's disease
Licensed content author	R. Takechi,S. Galloway,M.M.S. Pallebage-Gamarallage,J.C.L. Mamo
Licensed content date	September 2008
Licensed content volume number	9
Licensed content issue number	2
Number of pages	7
Start Page	19
End Page	25
Type of Use	reuse in a thesis/dissertation
Intended publisher of new work	other
Portion	full article
Format	both print and electronic
Are you the author of this Elsevier article?	Yes
Will you be translating?	No
Title of your thesis/dissertation	Enterocytic beta amyloid-lipoprotein homeostasis: Implications for Alzheimer's disease risk
Expected completion date	Oct 2014
Estimated size (number of pages)	220
Elsevier VAT number	GB 494 6272 12
Price	0.00 USD
VAT/Local Sales Tax	0.00 USD / 0.00 GBP
Total	0.00 USD
Terms and Conditions	

INTRODUCTION

1. The publisher for this copyrighted material is Elsevier. By clicking "accept" in connection with completing this licensing transaction, you agree that the following terms and conditions apply to this transaction (along with the Billing and Payment terms and conditions established by Copyright Clearance Center, Inc. ("CCC"), at the time that you opened your Rightslink account and that are

available at any time at http://myaccount.copyright.com).

GENERAL TERMS

2. Elsevier hereby grants you permission to reproduce the aforementioned material subject to the terms and conditions indicated.

3. Acknowledgement: If any part of the material to be used (for example, figures) has appeared in our publication with credit or acknowledgement to another source, permission must also be sought from that source. If such permission is not obtained then that material may not be included in your publication/copies. Suitable acknowledgement to the source must be made, either as a footnote or in a reference list at the end of your publication, as follows:

"Reprinted from Publication title, Vol /edition number, Author(s), Title of article / title of chapter, Pages No., Copyright (Year), with permission from Elsevier [OR APPLICABLE SOCIETY COPYRIGHT OWNER]." Also Lancet special credit - "Reprinted from The Lancet, Vol. number, Author(s), Title of article, Pages No., Copyright (Year), with permission from Elsevier."

4. Reproduction of this material is confined to the purpose and/or media for which permission is hereby given.

5. Altering/Modifying Material: Not Permitted. However figures and illustrations may be altered/adapted minimally to serve your work. Any other abbreviations, additions, deletions and/or any other alterations shall be made only with prior written authorization of Elsevier Ltd. (Please contact Elsevier at permissions@elsevier.com)

6. If the permission fee for the requested use of our material is waived in this instance, please be advised that your future requests for Elsevier materials may attract a fee.

7. Reservation of Rights: Publisher reserves all rights not specifically granted in the combination of (i) the license details provided by you and accepted in the course of this licensing transaction, (ii) these terms and conditions and (iii) CCC's Billing and Payment terms and conditions.

8. License Contingent Upon Payment: While you may exercise the rights licensed immediately upon issuance of the license at the end of the licensing process for the transaction, provided that you have disclosed complete and accurate details of your proposed use, no license is finally effective unless and until full payment is received from you (either by publisher or by CCC) as provided in CCC's Billing and Payment terms and conditions. If full payment is not received on a timely basis, then any license preliminarily granted shall be deemed automatically revoked and shall be void as if never granted. Further, in the event that you breach any of these terms and conditions or any of CCC's Billing and Payment terms and conditions, the license is automatically revoked and shall be void as if never granted. Use of materials as described in a revoked license, as well as any use of the materials beyond the scope of an unrevoked license, may constitute copyright infringement and publisher reserves the right to take any and all action to protect its copyright in the materials.

9. Warranties: Publisher makes no representations or warranties with respect to the licensed material.

10. Indemnity: You hereby indemnify and agree to hold harmless publisher and CCC, and their respective officers, directors, employees and agents, from and against any and all claims arising out

of your use of the licensed material other than as specifically authorized pursuant to this license.

11. No Transfer of License: This license is personal to you and may not be sublicensed, assigned, or transferred by you to any other person without publisher's written permission.

12. No Amendment Except in Writing: This license may not be amended except in a writing signed by both parties (or, in the case of publisher, by CCC on publisher's behalf).

13. Objection to Contrary Terms: Publisher hereby objects to any terms contained in any purchase order, acknowledgment, check endorsement or other writing prepared by you, which terms are inconsistent with these terms and conditions or CCC's Billing and Payment terms and conditions. These terms and conditions, together with CCC's Billing and Payment terms and conditions (which are incorporated herein), comprise the entire agreement between you and publisher (and CCC) concerning this licensing transaction. In the event of any conflict between your obligations established by these terms and conditions and those established by CCC's Billing and Payment terms and conditions, these terms and conditions shall control.

14. Revocation: Elsevier or Copyright Clearance Center may deny the permissions described in this License at their sole discretion, for any reason or no reason, with a full refund payable to you. Notice of such denial will be made using the contact information provided by you. Failure to receive such notice will not alter or invalidate the denial. In no event will Elsevier or Copyright Clearance Center be responsible or liable for any costs, expenses or damage incurred by you as a result of a denial of your permission request, other than a refund of the amount(s) paid by you to Elsevier and/or Copyright Clearance Center for denied permissions.

LIMITED LICENSE

The following terms and conditions apply only to specific license types:

15. **Translation**: This permission is granted for non-exclusive world **English** rights only unless your license was granted for translation rights. If you licensed translation rights you may only translate this content into the languages you requested. A professional translator must perform all translations and reproduce the content word for word preserving the integrity of the article. If this license is to re-use 1 or 2 figures then permission is granted for non-exclusive world rights in all languages.

16. **Posting licensed content on any Website**: The following terms and conditions apply as follows: Licensing material from an Elsevier journal: All content posted to the web site must maintain the copyright information line on the bottom of each image; A hyper-text must be included to the Homepage of the journal from which you are licensing at http://www.sciencedirect.com/science/journal/xxxxx or the Elsevier homepage for books at http://www.elsevier.com; Central Storage: This license does not include permission for a scanned version of the material to be stored in a central repository such as that provided by Heron/XanEdu.

Licensing material from an Elsevier book: A hyper-text link must be included to the Elsevier homepage at http://www.elsevier.com . All content posted to the web site must maintain the copyright information line on the bottom of each image.

Posting licensed content on Electronic reserve: In addition to the above the following clauses are applicable: The web site must be password-protected and made available only to bona fide students registered on a relevant course. This permission is granted for 1 year only. You may obtain a new

license for future website posting.

For journal authors: the following clauses are applicable in addition to the above: Permission granted is limited to the author accepted manuscript version* of your paper.

***Accepted Author Manuscript (AAM) Definition:** An accepted author manuscript (AAM) is the author's version of the manuscript of an article that has been accepted for publication and which may include any author-incorporated changes suggested through the processes of submission processing, peer review, and editor-author communications. AAMs do not include other publisher value-added contributions such as copy-editing, formatting, technical enhancements and (if relevant) pagination.

You are not allowed to download and post the published journal article (whether PDF or HTML, proof or final version), nor may you scan the printed edition to create an electronic version. A hyper-text must be included to the Homepage of the journal from which you are licensing at http://www.sciencedirect.com/science/journal/xxxxx. As part of our normal production process, you will receive an e-mail notice when your article appears on Elsevier's online service ScienceDirect (www.sciencedirect.com). That e-mail will include the article's Digital Object Identifier (DOI). This number provides the electronic link to the published article and should be included in the posting of your personal version. We ask that you wait until you receive this e-mail and have the DOI to do any posting.

Posting to a repository: Authors may post their AAM immediately to their employer's institutional repository for internal use only and may make their manuscript publically available after the journal-specific embargo period has ended.

Please also refer to Elsevier's Article Posting Policy for further information.

18. **For book authors** the following clauses are applicable in addition to the above: Authors are permitted to place a brief summary of their work online only.. You are not allowed to download and post the published electronic version of your chapter, nor may you scan the printed edition to create an electronic version. **Posting to a repository:** Authors are permitted to post a summary of their chapter only in their institution's repository.

20. **Thesis/Dissertation**: If your license is for use in a thesis/dissertation your thesis may be submitted to your institution in either print or electronic form. Should your thesis be published commercially, please reapply for permission. These requirements include permission for the Library and Archives of Canada to supply single copies, on demand, of the complete thesis and include permission for UMI to supply single copies, on demand, of the complete thesis. Should your thesis be published commercially, please reapply for permission.

Elsevier Open Access Terms and Conditions

Elsevier publishes Open Access articles in both its Open Access journals and via its Open Access articles option in subscription journals.

Authors publishing in an Open Access journal or who choose to make their article Open Access in an Elsevier subscription journal select one of the following Creative Commons user licenses, which define how a reader may reuse their work: Creative Commons Attribution License (CC BY), Creative Commons Attribution – Non Commercial - ShareAlike (CC BY NC SA) and Creative Commons

Attribution – Non Commercial – No Derivatives (CC BY NC ND)

Terms & Conditions applicable to all Elsevier Open Access articles:

Any reuse of the article must not represent the author as endorsing the adaptation of the article nor should the article be modified in such a way as to damage the author's honour or reputation.

The author(s) must be appropriately credited.

If any part of the material to be used (for example, figures) has appeared in our publication with credit or acknowledgement to another source it is the responsibility of the user to ensure their reuse complies with the terms and conditions determined by the rights holder.

Additional Terms & Conditions applicable to each Creative Commons user license:

CC BY: You may distribute and copy the article, create extracts, abstracts, and other revised versions, adaptations or derivative works of or from an article (such as a translation), to include in a collective work (such as an anthology), to text or data mine the article, including for commercial purposes without permission from Elsevier

CC BY NC SA: For non-commercial purposes you may distribute and copy the article, create extracts, abstracts and other revised versions, adaptations or derivative works of or from an article (such as a translation), to include in a collective work (such as an anthology), to text and data mine the article and license new adaptations or creations under identical terms without permission from Elsevier

CC BY NC ND: For non-commercial purposes you may distribute and copy the article and include it in a collective work (such as an anthology), provided you do not alter or modify the article, without permission from Elsevier

Any commercial reuse of Open Access articles published with a CC BY NC SA or CC BY NC ND license requires permission from Elsevier and will be subject to a fee.

Commercial reuse includes:

· Promotional purposes (advertising or marketing)

· Commercial exploitation (e.g. a product for sale or loan)

· Systematic distribution (for a fee or free of charge)

Please refer to Elsevier's Open Access Policy for further information.

21. **Other Conditions**:

v1.6

Questions? customercare@copyright.com or +1-855-239-3415 (toll free in the US) or +1-978-646-2777.

Gratis licenses (referencing $0 in the Total field) are free. Please retain this printable license for your reference. No payment is required.

Article 2:

Pallebage-Gamarallage, M. M., **S. Galloway**, R. Johnsen, L. Jian, S. Dhaliwal, and J. C. Mamo. "The Effect of Exogenous Cholesterol and Lipid-Modulating Agents on

Enterocytic Amyloid-Beta Abundance." [In eng]. *Br J Nutr* 101, no. 3 (Feb 2009): 340-7.

Menuka M. Pallebage-Gamarallage contributed to experimental protocols, data collection and drafting of the manuscript. Susan Galloway assisted in data collection and appraisal of the manuscript. Russell D. Johnsen assisted in method development and the review of the manuscript. Le Jian contributed to appraisal of the manuscript. Satvinder Dhaliwal provided statistical analysis of results and assisted in study design. John C. Mamo was instrumental in the design and funding of the project, experiment design, collection of data, and writing of the manuscript.

NON EXCLUSIVE LICENCE/INVOICE TO USE COPYRIGHT MATERIAL

Attention:
Susan Galloway
Susan.galloway@postgrad.curtin.edu.au
159 Elderberry Drive
South Lake
Perth WA 6164

CAMBRIDGE UNIVERSITY PRESS

Australia & New Zealand
477 Williamstown Road
Port Melbourne
Victoria 3207
Australia

Correspondence
Private Bag 31
Port Melbourne
Victoria 3207
Australia

www.cambridge.edu.au

Telephone +61 (03) 8671 1400
Fax +61 (03) 9676 9966
Email info@cambridge.edu.au
ABN 28 508 204 178

Friday 5 September 2014

OUR REFERENCE

CUP Ref:	AUS15092014
ISSN:	0007-1145
Journal:	*British Journal of Nutrition*
Title:	'The effect of exogenous cholesterol and lipid-modulating agents on enterocytic amyloid-β abundance'
Author/s:	Menuka M. Pallebage-Gamarallage, Susan Galloway, Russell Johnsen, Le Jian, Satvinder Dhaliwal and John C. L. Mamo
Pub info:	Volume 101 / Issue 03 / February 2009, pp 340-347
Title:	'Differential effects of dietary fatty acids on the cerebral distribution of plasma-derived apo B lipoproteins with amyloid-β'
Author/s:	Ryusuke Takechi, Susan Galloway, Menuka M. S. Pallebage-Gamarallage, Cheryl L. Wellington, Russell D. Johnsen, Satvinder S. Dhaliwal and John C. L. Mamo
Pub info:	Volume 103 / Issue 05 / March 2010, pp 652-662
Your use:	As per notifications dated 30/08/2014

Thank you for your recent permission request to include your above listed articles in your doctoral thesis for Curtin University of Technology in Perth, WA. Upon completion, the thesis and the above articles included, will be made available in hard-copy in the Curtin Library and in digital form on the Internet via the Australian Digital Thesis Program.

Permission is granted for this specific use on the understanding that you have checked that we do not acknowledge another source for this material and provided the posting includes a prominent statement of the full bibliographical details of the article and a link to the Cambridge website address for the journal.

Please ensure full acknowledgements:

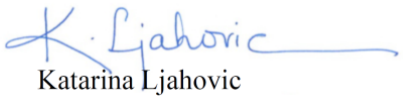
Author/s, title, journal, publication date, copyright notice of the journal and Cambridge University Press.

Please include a link to the Cambridge website for the journal articles:

http://journals.cambridge.org/action/displayAbstract?fromPage=online&aid=3324300&fileId=S0007114508012269

http://journals.cambridge.org/action/displayAbstract?fromPage=online&aid=7278500&fulltextType=RA&fileId=S0007114509992194

Signed:



Katarina Ljahovic
Permissions Controller
Cambridge University Press
Australia and New Zealand

Article 3:

Pallebage-Gamarallage, M. M., **S. Galloway**, R. Takechi, S. Dhaliwal, and J. C. Mamo. "Probucol Suppresses Enterocytic Accumulation of Amyloid-Beta Induced by Saturated Fat and Cholesterol Feeding." [In eng]. *Lipids* 47, no. 1 (Jan 2012): 27-34.

Menuka M. Pallebage-Gamarallage designed and undertook the experimental protocols including tissue collection, collating data, analysis of data, and preparation of the research article. Susan Galloway and Ryusuke Takechi contributed to tissue collection and Satvinder Dhaliwal assisted in statistical interpretation of data. John C. Mamo assisted in study design and interpretation of data and contributed to the review of the final manuscript.

SPRINGER LICENSE
TERMS AND CONDITIONS

Sep 23, 2014

This is a License Agreement between Susan Galloway ("You") and Springer ("Springer") provided by Copyright Clearance Center ("CCC"). The license consists of your order details, the terms and conditions provided by Springer, and the payment terms and conditions.

All payments must be made in full to CCC. For payment instructions, please see information listed at the bottom of this form.

License Number	3462980247772
License date	Sep 06, 2014
Order Content Publisher	Springer
Order Content Publication	Lipids
Order Content Title	Probucol Suppresses Enterocytic Accumulation of Amyloid-β Induced by Saturated Fat and Cholesterol Feeding
Order Content Author	Menuka M. Pallebage-Gamarallage
Order Content Date	Jan 1, 2011
Volume number	47
Issue number	1
Type of Use	Thesis/Dissertation
Portion	Full text
Number of copies	5
Author of this Springer article	No
Order reference number	None
Title of your thesis / dissertation	Enterocytic beta amyloid-lipoprotein homeostasis: Implications for Alzheimer's disease risk
Expected completion date	Oct 2014
Estimated size(pages)	220
Total	0.00 USD

Terms and Conditions

Introduction

The publisher for this copyrighted material is Springer Science + Business Media. By clicking "accept" in connection with completing this licensing transaction, you agree that the following terms and conditions apply to this transaction (along with the Billing and Payment terms and conditions established by Copyright Clearance Center, Inc. ("CCC"), at the time that you opened your Rightslink account and that are available at any time at http://myaccount.copyright.com).

Limited License

With reference to your request to reprint in your thesis material on which Springer Science and Business Media control the copyright, permission is granted, free of charge, for the use indicated in your enquiry.

out of your use of the licensed material other than as specifically authorized pursuant to this license.

No Transfer of License
This license is personal to you and may not be sublicensed, assigned, or transferred by you to any other person without Springer Science + Business Media's written permission.

No Amendment Except in Writing
This license may not be amended except in a writing signed by both parties (or, in the case of Springer Science + Business Media, by CCC on Springer Science + Business Media's behalf).

Objection to Contrary Terms
Springer Science + Business Media hereby objects to any terms contained in any purchase order, acknowledgment, check endorsement or other writing prepared by you, which terms are inconsistent with these terms and conditions or CCC's Billing and Payment terms and conditions. These terms and conditions, together with CCC's Billing and Payment terms and conditions (which are incorporated herein), comprise the entire agreement between you and Springer Science + Business Media (and CCC) concerning this licensing transaction. In the event of any conflict between your obligations established by these terms and conditions and those established by CCC's Billing and Payment terms and conditions, these terms and conditions shall control.

Jurisdiction
All disputes that may arise in connection with this present License, or the breach thereof, shall be settled exclusively by arbitration, to be held in The Netherlands, in accordance with Dutch law, and to be conducted under the Rules of the 'Netherlands Arbitrage Instituut' (Netherlands Institute of Arbitration).***OR:***

All disputes that may arise in connection with this present License, or the breach thereof, shall be settled exclusively by arbitration, to be held in the Federal Republic of Germany, in accordance with German law.

Other terms and conditions:

v1.3

Questions? customercare@copyright.com or +1-855-239-3415 (toll free in the US) or +1-978-646-2777.

Gratis licenses (referencing $0 in the Total field) are free. Please retain this printable license for your reference. No payment is required.

Article 4:

Takechi, R., **S. Galloway**, M. M. Pallebage-Gamarallage, C. L. Wellington, R. D. Johnsen, S. S. Dhaliwal, and J. C. Mamo. "Differential Effects of Dietary Fatty Acids on the Cerebral Distribution of Plasma-Derived Apo B Lipoproteins with Amyloid-Beta." [In eng]. *Br J Nutr* 103, no. 5 (Mar 2010): 652-62.

Takechi Ryusuke led the experiments and was responsible for the study design, methodology, data collection and interpretation and drafting of the manuscript. Susan Galloway contributed to study design, maintenance of animals, tissue collection and manuscript appraisal. Menuka M. Pallebage-Gamarallage assisted with animal maintenance, data collection and manuscript appraisal. Cheryl L. Wellington provided tissue samples as well as reviewing the draft manuscript. Russell D. Johnsen provided assistance with development of methods. Satvinder S. Dhaliwal assisted with statistical analysis of results. John C. Mamo contributed to the design of the study, interpretation of data and critical appraisal of the manuscript.

NON EXCLUSIVE LICENCE/INVOICE TO USE COPYRIGHT MATERIAL

Attention:
Susan Galloway
Susan.galloway@postgrad.curtin.edu.au
159 Elderberry Drive
South Lake
Perth WA 6164

CAMBRIDGE UNIVERSITY PRESS

Australia & New Zealand
477 Williamstown Road
Port Melbourne
Victoria 3207
Australia

Correspondence
Private Bag 31
Port Melbourne
Victoria 3207
Australia

www.cambridge.edu.au

Telephone +61 (03) 8671 1400
Fax +61 (03) 9676 9966
Email info@cambridge.edu.au
ABN 28 508 204 178

Friday 5 September 2014

OUR REFERENCE

CUP Ref:	AUS15092014
ISSN:	0007-1145
Journal:	*British Journal of Nutrition*
Title:	'The effect of exogenous cholesterol and lipid-modulating agents on enterocytic amyloid-β abundance'
Author/s:	Menuka M. Pallebage-Gamarallage, Susan Galloway, Russell Johnsen, Le Jian, Satvinder Dhaliwal and John C. L. Mamo
Pub info:	Volume 101 / Issue 03 / February 2009, pp 340-347
Title:	'Differential effects of dietary fatty acids on the cerebral distribution of plasma-derived apo B lipoproteins with amyloid-β'
Author/s:	Ryusuke Takechi, Susan Galloway, Menuka M. S. Pallebage-Gamarallage, Cheryl L. Wellington, Russell D. Johnsen, Satvinder S. Dhaliwal and John C. L. Mamo
Pub info:	Volume 103 / Issue 05 / March 2010, pp 652-662
Your use:	As per notifications dated 30/08/2014

Thank you for your recent permission request to include your above listed articles in your doctoral thesis for Curtin University of Technology in Perth, WA. Upon completion, the thesis and the above articles included, will be made available in hard-copy in the Curtin Library and in digital form on the Internet via the Australian Digital Thesis Program.

Permission is granted for this specific use on the understanding that you have checked that we do not acknowledge another source for this material and provided the posting includes a prominent statement of the full bibliographical details of the article and a link to the Cambridge website address for the journal.

Please ensure full acknowledgements:

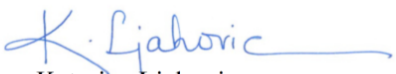
Author/s, title, journal, publication date, copyright notice of the journal and Cambridge University Press.

Please include a link to the Cambridge website for the journal articles:

http://journals.cambridge.org/action/displayAbstract?fromPage=online&aid=3324300&fileId=S0007114508012269

http://journals.cambridge.org/action/displayAbstract?fromPage=online&aid=7278500&fulltextType=RA&fileId=S0007114509992194

Signed:



Katarina Ljahovic
Permissions Controller
Cambridge University Press
Australia and New Zealand

Article 5:

Takechi R, **Galloway S**, Pallebage-Gamarallage MM, Lam V, Mamo JCL. (2009) Dietary fats, cerebrovascular integrity and Alzheimer's disease risk. *Prog Lipid Res*. 49, 159-170

Ryusuke Takechi was responsible for the review and interpretation of literature, and writing of the manuscript. Susan Galloway, Menuka M. Pallebage-Gamarallage and Virginie Lam assisted in the generation of images and review of the draft manuscript. John C. Mamo was involved in discussion of ideas and appraisal of current literature.

ELSEVIER LICENSE
TERMS AND CONDITIONS

Sep 23, 2014

This is a License Agreement between Susan Galloway ("You") and Elsevier ("Elsevier") provided by Copyright Clearance Center ("CCC"). The license consists of your order details, the terms and conditions provided by Elsevier, and the payment terms and conditions.

All payments must be made in full to CCC. For payment instructions, please see information listed at the bottom of this form.

Supplier	Elsevier Limited The Boulevard,Langford Lane Kidlington,Oxford,OX5 1GB,UK
Registered Company Number	1982084
Customer name	Susan Galloway
Customer address	159 Elderberry Dr, Perth, None 6164
License number	3461960727925
License date	Sep 04, 2014
Licensed content publisher	Elsevier
Licensed content publication	Progress in Lipid Research
Licensed content title	Dietary fats, cerebrovasculature integrity and Alzheimer's disease risk
Licensed content author	R. Takechi,S. Galloway,M.M.S. Pallebage-Gamarallage,V. Lam,J.C.L. Mamo
Licensed content date	April 2010
Licensed content volume number	49
Licensed content issue number	2
Number of pages	12
Start Page	159
End Page	170
Type of Use	reuse in a thesis/dissertation
Intended publisher of new work	other
Portion	full article
Format	both print and electronic
Are you the author of this Elsevier article?	Yes
Will you be translating?	No
Title of your thesis/dissertation	Enterocytic beta amyloid-lipoprotein homeostasis: Implications for Alzheimer's disease risk
Expected completion date	Oct 2014
Estimated size (number of pages)	220
Elsevier VAT number	GB 494 6272 12

Price	0.00 USD
VAT/Local Sales Tax	0.00 USD / 0.00 GBP
Total	0.00 USD

Terms and Conditions

INTRODUCTION

1. The publisher for this copyrighted material is Elsevier. By clicking "accept" in connection with completing this licensing transaction, you agree that the following terms and conditions apply to this transaction (along with the Billing and Payment terms and conditions established by Copyright Clearance Center, Inc. ("CCC"), at the time that you opened your Rightslink account and that are available at any time at http://myaccount.copyright.com).

GENERAL TERMS

2. Elsevier hereby grants you permission to reproduce the aforementioned material subject to the terms and conditions indicated.

3. Acknowledgement: If any part of the material to be used (for example, figures) has appeared in our publication with credit or acknowledgement to another source, permission must also be sought from that source. If such permission is not obtained then that material may not be included in your publication/copies. Suitable acknowledgement to the source must be made, either as a footnote or in a reference list at the end of your publication, as follows:

"Reprinted from Publication title, Vol /edition number, Author(s), Title of article / title of chapter, Pages No., Copyright (Year), with permission from Elsevier [OR APPLICABLE SOCIETY COPYRIGHT OWNER]." Also Lancet special credit - "Reprinted from The Lancet, Vol. number, Author(s), Title of article, Pages No., Copyright (Year), with permission from Elsevier."

4. Reproduction of this material is confined to the purpose and/or media for which permission is hereby given.

5. Altering/Modifying Material: Not Permitted. However figures and illustrations may be altered/adapted minimally to serve your work. Any other abbreviations, additions, deletions and/or any other alterations shall be made only with prior written authorization of Elsevier Ltd. (Please contact Elsevier at permissions@elsevier.com)

6. If the permission fee for the requested use of our material is waived in this instance, please be advised that your future requests for Elsevier materials may attract a fee.

7. Reservation of Rights: Publisher reserves all rights not specifically granted in the combination of (i) the license details provided by you and accepted in the course of this licensing transaction, (ii) these terms and conditions and (iii) CCC's Billing and Payment terms and conditions.

8. License Contingent Upon Payment: While you may exercise the rights licensed immediately upon issuance of the license at the end of the licensing process for the transaction, provided that you have disclosed complete and accurate details of your proposed use, no license is finally effective unless and until full payment is received from you (either by publisher or by CCC) as provided in CCC's Billing and Payment terms and conditions. If full payment is not received on a timely basis, then any license preliminarily granted shall be deemed automatically revoked and shall be void as if never granted. Further, in the event that you breach any of these terms and conditions or any of CCC's

Billing and Payment terms and conditions, the license is automatically revoked and shall be void as if never granted. Use of materials as described in a revoked license, as well as any use of the materials beyond the scope of an unrevoked license, may constitute copyright infringement and publisher reserves the right to take any and all action to protect its copyright in the materials.

9. Warranties: Publisher makes no representations or warranties with respect to the licensed material.

10. Indemnity: You hereby indemnify and agree to hold harmless publisher and CCC, and their respective officers, directors, employees and agents, from and against any and all claims arising out of your use of the licensed material other than as specifically authorized pursuant to this license.

11. No Transfer of License: This license is personal to you and may not be sublicensed, assigned, or transferred by you to any other person without publisher's written permission.

12. No Amendment Except in Writing: This license may not be amended except in a writing signed by both parties (or, in the case of publisher, by CCC on publisher's behalf).

13. Objection to Contrary Terms: Publisher hereby objects to any terms contained in any purchase order, acknowledgment, check endorsement or other writing prepared by you, which terms are inconsistent with these terms and conditions or CCC's Billing and Payment terms and conditions. These terms and conditions, together with CCC's Billing and Payment terms and conditions (which are incorporated herein), comprise the entire agreement between you and publisher (and CCC) concerning this licensing transaction. In the event of any conflict between your obligations established by these terms and conditions and those established by CCC's Billing and Payment terms and conditions, these terms and conditions shall control.

14. Revocation: Elsevier or Copyright Clearance Center may deny the permissions described in this License at their sole discretion, for any reason or no reason, with a full refund payable to you. Notice of such denial will be made using the contact information provided by you. Failure to receive such notice will not alter or invalidate the denial. In no event will Elsevier or Copyright Clearance Center be responsible or liable for any costs, expenses or damage incurred by you as a result of a denial of your permission request, other than a refund of the amount(s) paid by you to Elsevier and/or Copyright Clearance Center for denied permissions.

LIMITED LICENSE

The following terms and conditions apply only to specific license types:

15. **Translation**: This permission is granted for non-exclusive world **English** rights only unless your license was granted for translation rights. If you licensed translation rights you may only translate this content into the languages you requested. A professional translator must perform all translations and reproduce the content word for word preserving the integrity of the article. If this license is to re-use 1 or 2 figures then permission is granted for non-exclusive world rights in all languages.

16. **Posting licensed content on any Website**: The following terms and conditions apply as follows: Licensing material from an Elsevier journal: All content posted to the web site must maintain the copyright information line on the bottom of each image; A hyper-text must be included to the Homepage of the journal from which you are licensing at http://www.sciencedirect.com/science/journal/xxxxx or the Elsevier homepage for books at http://www.elsevier.com; Central Storage: This license does not include permission for a scanned

version of the material to be stored in a central repository such as that provided by Heron/XanEdu.

Licensing material from an Elsevier book: A hyper-text link must be included to the Elsevier homepage at http://www.elsevier.com . All content posted to the web site must maintain the copyright information line on the bottom of each image.
Posting licensed content on Electronic reserve: In addition to the above the following clauses are applicable: The web site must be password-protected and made available only to bona fide students registered on a relevant course. This permission is granted for 1 year only. You may obtain a new license for future website posting.

For journal authors: the following clauses are applicable in addition to the above: Permission granted is limited to the author accepted manuscript version* of your paper.

***Accepted Author Manuscript (AAM) Definition:** An accepted author manuscript (AAM) is the author's version of the manuscript of an article that has been accepted for publication and which may include any author-incorporated changes suggested through the processes of submission processing, peer review, and editor-author communications. AAMs do not include other publisher value-added contributions such as copy-editing, formatting, technical enhancements and (if relevant) pagination.

You are not allowed to download and post the published journal article (whether PDF or HTML, proof or final version), nor may you scan the printed edition to create an electronic version. A hyper-text must be included to the Homepage of the journal from which you are licensing at http://www.sciencedirect.com/science/journal/xxxxx. As part of our normal production process, you will receive an e-mail notice when your article appears on Elsevier's online service ScienceDirect (www.sciencedirect.com). That e-mail will include the article's Digital Object Identifier (DOI). This number provides the electronic link to the published article and should be included in the posting of your personal version. We ask that you wait until you receive this e-mail and have the DOI to do any posting.

Posting to a repository: Authors may post their AAM immediately to their employer's institutional repository for internal use only and may make their manuscript publically available after the journal-specific embargo period has ended.

Please also refer to Elsevier's Article Posting Policy for further information.

18. **For book authors** the following clauses are applicable in addition to the above: Authors are permitted to place a brief summary of their work online only.. You are not allowed to download and post the published electronic version of your chapter, nor may you scan the printed edition to create an electronic version. **Posting to a repository:** Authors are permitted to post a summary of their chapter only in their institution's repository.

20. **Thesis/Dissertation**: If your license is for use in a thesis/dissertation your thesis may be submitted to your institution in either print or electronic form. Should your thesis be published commercially, please reapply for permission. These requirements include permission for the Library and Archives of Canada to supply single copies, on demand, of the complete thesis and include permission for UMI to supply single copies, on demand, of the complete thesis. Should your thesis be published commercially, please reapply for permission.

Elsevier Open Access Terms and Conditions

21. **Other Conditions**:

v1.6

Questions? customercare@copyright.com or +1-855-239-3415 (toll free in the US) or +1-978-646-2777.

Gratis licenses (referencing $0 in the Total field) are free. Please retain this printable license for your reference. No payment is required.

I, Susan Galloway here confirm that I made the contributions to the publications included in this thesis as stated above.

_____

I, Ryusuke Takechi, here confirm that I made the contributions to the publications included in this thesis as stated above and agreed to give permission to use these publications as a part of Susan Galloway's thesis.

_____

I, Menuka Pallebage-Gamarallage, here confirm that I made the contributions to the publications included in this thesis as stated above. I agree to give permission to use these publications as a part of Susan Galloway's thesis.

_____

I, Russell Johnsen, here confirm that I made the contributions to the publications included in this thesis as stated above and agreed to give permission to use these publications as a part of Susan Galloway's thesis.

_____

I, Le Jian, here confirm that I made the contributions to the publications included in this thesis as stated above and agreed to give permission to use these publications as a part of Susan Galloway's thesis.

_____

I, Satvinder Dhaliwal, here confirm that I made the contributions to the publications included in this thesis as stated above and agreed to give permission to use these publications as a part of Susan Galloway's thesis.

_____

I, Stewart Chew, here confirm that I made the contributions to the publications included in this thesis as stated above and agreed to give permission to use these publications as a part of Susan Galloway's thesis.

_____

I, Cheryl Wellington, here confirm that I made the contributions to the publications included in this thesis as stated above and agreed to give permission to use these publications as a part of Susan Galloway's thesis.

_____

I, Virginie Lam, here confirm that I made the contributions to the publications included in this thesis as stated above and agreed to give permission to use these publications as a part of Susan Galloway's thesis.

_____

I, John Mamo, here confirm that I made the contributions to the publications included in this thesis as stated above. I agree to give permission to use these publications as a part of Susan Galloway's thesis.

_____

Appendix B: Additional co-authored manuscripts

This thesis contains additional publications which are not a part of the main body and main ideas of the thesis. These articles do however, support the broad hypothesis and provide additional insights from alternative perspective. The results of findings are published in internationally recognised journals.

This thesis is supported by the following co-authored journal articles:

Article 1:

Takechi R, **Galloway S**, Pallebage-Gamarallage MM, Wellington C, Mamo JC. (2008) Chylomicron amyloid-beta in the aetiology of Alzheimer's disease. *Atheroscler Suppl.* 9, 19-25.

Article 2:

Pallebage-Gamarallage, M. M., **S. Galloway**, R. Johnsen, L. Jian, S. Dhaliwal, and J. C. Mamo. "The Effect of Exogenous Cholesterol and Lipid-Modulating Agents on Enterocytic Amyloid-Beta Abundance." [In eng]. *Br J Nutr* 101, no. 3 (Feb 2009): 340-7.

Article 3:

Pallebage-Gamarallage, M. M., **S. Galloway**, R. Takechi, S. Dhaliwal, and J. C. Mamo. "Probucol Suppresses Enterocytic Accumulation of Amyloid-Beta Induced by Saturated Fat and Cholesterol Feeding." [In eng]. *Lipids* 47, no. 1 (Jan 2012): 27-34.

Article 4:

Takechi, R., **S. Galloway**, M. M. Pallebage-Gamarallage, C. L. Wellington, R. D. Johnsen, S. S. Dhaliwal, and J. C. Mamo. "Differential Effects of Dietary Fatty Acids on the Cerebral Distribution of Plasma-Derived Apo B Lipoproteins with Amyloid-Beta." [In eng]. *Br J Nutr* 103, no. 5 (Mar 2010): 652-62.

Article 5:

Takechi R, **Galloway S**, Pallebage-Gamarallage MM, Lam V, Mamo JCL. (2009) Dietary fats, cerebrovascular integrity and Alzheimer's disease risk. *Prog Lipid Res*. 49, 159-170.

Article 1:

Takechi R, **Galloway S**, Pallebage-Gamarallage MM, Wellington C, Mamo JC. (2008) Chylomicron amyloid-beta in the aetiology of Alzheimer's disease. *Atheroscler Suppl.* 9, 19-25 (Review)

Synopsis

This review article presents current research regarding dietary fats and AD and provides an alternate novel perspective on pathological mechanisms involved.

A number of epidemiological and animal studies show high intake of fats and cholesterol correlates with increased risk of developing AD via mechanisms related to the metabolism of Aβ (Kalmijn et al. 1997, Petot et al. 2004, Refolo et al. 2000, Sparks et al. 1994), however, the exact mechanisms remain unclear. Although Aβ, the derivative of precursor APP, has been shown to specifically cause a pathological cascade of events leading to AD, the origin of this crucial protein remains a subject of debate.

One pathological feature that is common amongst AD-affected individuals is the presence of Aβ within the blood vessels of the brain and in many cases, damage to the BBB (Kalaria 1992, Wisniewski et al. 1997). The transport of Aβ across the BBB has been implicated and plasma Aβ can contribute to significant cerebral load (Deane et al. 2005) however currently, research into peripherally derived Aβ is limited. In this study we reported the expression of Aβ within small intestine enterocytes, which are responsible for absorption and transport of dietary fats. In addition, expression of Aβ was significantly enhanced by high fat feeding and abolished by fasting (Galloway et al. 2007). These findings provide an alternative view in understanding the possible pathological influence of dietary fats on the development of AD. Although cerebral amyloidosis was not observed in wild type mice involved in this study, LaRue et al. (2004) showed high-fat feeding induced an 8-fold increase in the rate of peripheral Aβ transport into the brain.

A number of studies have shown that acute and chronic exposure of cerebrovasculature to Aβ can result in damage to endothelial cells of the tunica intima (Thomas et al. 1997). In this study, peripheral administration of Aβ resulted in entrapment of Aβ within blood vessels and transportation into brain (Maness et al. 1994, Martel et al. 1996, Zlokovic et al. 1993) and chronic exposure to peripheral Aβ was shown to damage blood vessels and activate the brain's immune response (Su et al. 1999). Pathological consequences of damaged BBB could be increased transport of Aβ into the brain and/or decreased efflux of Aβ from the brain.

Section 3 and 4 of the article discusses the production and association of enterocytic Aβ with CMs. Our study in wild-type C57/BL6J mice shows the distinct presence of Aβ proteins within the perinuclear location of enterocytes and other studies indicate Aβ co-localises with enterocytic apo B and Golgi apparatus. Saturated fatty acids and UFAs feeding in animals show substantial expression of Aβ with apo B. The association of Aβ with apo B is presently unclear; however, the cysteine-rich motif of APP/Aβ shares homology with some lipoprotein receptors. As these domains can bind to apo E-containing lipoproteins with substantial affinity, it is reasonable to assume that endogenously produced Aβ can bind and associate with apo E-containing CMs. The presence of Aβ within ER/Golgi region suggests association of Aβ during lipidation of apo B; a process which produces primordial lipoproteins. Lipid substrate availability is crucial for lipidation of apo B and formation of CM molecules and high fat induced increases in enterocytic Aβ suggests involvement and secretion of Aβ in complex with lipoproteins (James et al. 2003).

Several lines of evidence support the binding and association of Aβ with intestinally derived lipoproteins. Clinical studies show increases in APP processing following ingestion of fatty meal and increased secretion of intestinal Aβ-lipoproteins into blood (Boyt et al. 1999). Analysis of plasma from probable AD subjects (Mamo et al. 2008) shows that the majority of plasma Aβ is associated with TRL fraction including CMs and their remnants. This study determined that Aβ40 was significantly increased in TRL fraction of AD subjects compared to cognitively intact controls. In addition, although the subjects analysed were normolipidemic, there were significant decreases in the rate of clearance of postprandial lipoproteins marked by apo B48. High levels of post-prandial apo B48 can indicate post-prandial

dyslipidemia, a condition where increases in dietary induced secretions of CMs are not sufficiently cleared (Smith et al. 1999).

In section 5, A transgenic mouse (APP/PS1) model of AD (mirrors cerebral AD pathology by over-expression of APP) shows significant increases in plasma Aβ with increases in plasma TGs, as well as TRLs. Interestingly, these observations were made prior to the onset of cerebral and cerebrovascular accumulation of Aβ. Further analysis of brain samples from APP/PS1 mice showed higher levels of IgG in plaques and increased occludin which indicates increased permeability of the BBB in these animals.

Section 6 links the role of apo E isoforms with current findings/knowledge in CM-Aβ metabolism. Interestingly, apo E4 present in 50 % of AD individuals and influences kinetics of CMs. Apolipoprotein E4 preferentially binds to CMs and VLDL remnants which indicate a possibly greater plasma concentration of Aβ not associated with apo E. Apolipoprotein E4-containing individuals contains more permeable BBB which makes them at greater risk towards increased influx of plasma Aβ.

Lastly, section 7 summarises the current understanding surrounding dietary fat induced intestinal production of Aβ-lipoprotein and BBB integrity. High SFA induced exacerbation of cerebral amyloid deposition could be linked to a build-up of CM-Aβ and with increased permeability of the BBB can result in accumulation of these toxic peptides in the brain. Finally, this article recommends prevention and therapy of AD via lipid modulating agents and dietary regulation.

ELSEVIER

Atherosclerosis Supplements 9 (2008) 19–25

ATHEROSCLEROSIS
SUPPLEMENTS

www.elsevier.com/locate/atherosclerosis

Chylomicron amyloid-beta in the aetiology of Alzheimer's disease

R. Takechi, S. Galloway, M.M.S. Pallebage-Gamarallage, J.C.L. Mamo *

Faculty of Health Sciences, Curtin University of Technology, ATN Centre for Metabolic Health and Fitness, Building 400, Bentley Campus, Perth, Western Australia 6102, Australia

Received 31 January 2008; received in revised form 12 March 2008; accepted 13 May 2008

Abstract

Alzheimer's disease is characterized by inflammatory proteinaceous deposits comprised principally of the protein amyloid-beta (Aβ). Presently, the origins of cerebral amyloid deposits are controversial, though pivotal for the prevention of Alzheimer's disease.

Recent evidence suggests that in blood, Aβ may serve as a regulating apoprotein of the triglyceride-rich-lipoproteins and we have found that the synthesis of Aβ in enterocytes and thereafter secretion as part of the chylomicron cascade is regulated by dietary fats.

It is our contention that chronically elevated plasma levels of Aβ in response to diets rich in saturated fats may lead to disturbances within the cerebrovasculature and exaggerated blood-to-brain delivery of circulating Aβ, thereby exacerbating amyloidosis. Consistent with this hypothesis we show that enterocytic Aβ is increased concomitant with apolipoprotein B48. Furthermore, cerebral extravasation of immunoglobulin G, a surrogate marker of plasma proteins is observed in a murine model of Alzheimer's disease maintained on a saturated-fat diet and there is diminished expression of occludin within the cerebrovasculature, an endothelial tight junction protein.

Keywords: Chylomicrons; Amyloid-beta; Alzheimer's disease; Blood–brain barrier

1. Cerebrovascular integrity in Alzheimer's disease

Based on epidemiologic studies, there are statistically significant correlations between the prevalence of Alzheimer's disease (AD) and diabetes, hypercholesterolemia, hypertension, hyperhomocysteinemia, dietary saturated fats, cholesterol, antioxidants, alcohol consumption, smoking, physical activity, atherosclerotic disease, and the plasma concentration of some hemostatic factors. Most of the risk factors found to be associated with AD are age-dependent, and the prevalence of AD increases with age. Therefore, the association could simply be attributed to aging. On the other hand, common pathogenetic mechanisms for diseases such as AD and atherosclerosis, such as inflammation and the generation of free radicals, suggest a causal link. If this is the case, the identification of modifiable risk factors for dementia becomes a research priority and early intervention aimed at reducing those risk factors by therapeutic imperative.

An accumulating body of evidence is consistent with the concept that the onset and progression of sporadic and late-onset AD is significantly influenced by lifestyle factors including nutrition. Several population studies in humans have found that high-fat diets are a positive risk factor [1,2], and high-fat feeding markedly exacerbates Alzheimer's-like cerebral pathology in animal models of AD [3,4]. The mechanisms for the high-fat diet/AD link are presently unclear, but we will present in this article a novel hypothesis that may explain this effect. We contend that further studies are urgently needed to delineate the relationship between diet and AD. Indeed, by 2030 the expected global health burden for dementia will exceed treatment of cancers by 30% and will be equivalent to the combined costs for the prevention and treatment of cardiovascular disease.

The cerebrovasculature in subjects with AD shows pathological alterations including vascular endothelial and smooth muscle cell proliferation [5]. Blood plasma proteins have been detected in the parenchyma of AD brains [6,7] and inflammatory sequelae are commonly reported [8,9], observations that are consistent with breakdown of the blood–brain barrier (BBB). Despite the increasing evidence supportive of AD having an underlying vascular component, most research focuses on damage of neurons [10].

* Corresponding author. Tel.: +61 8 92667232; fax: +61 8 92662258.
E-mail address: J.Mamo@Curtin.edu.au (J.C.L. Mamo).

1567-5688/$ – see front matter © 2008 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.atherosclerosissup.2008.05.010

A major neuropathological marker of AD is amyloid-beta (Aβ) deposition in the cerebrovasculature. However, clarification of its putative role in the aetiology of AD has been more elusive. The pivotal question is whether cerebral and cerebrovascular Aβ is a final neurotoxic, common to all forms of AD; a toxic by-product of an independent primary metabolic lesion that is also neurotoxic; or an inert by-product of an independent primary neurotoxic reaction [11].

Derived from the amyloid precursor protein (APP), Aβ is the predominant component of amyloid plaque [12]. The putative source of cerebrovascular Aβ deposits in AD is controversial. There is little evidence for increased Aβ production in sporadic and late-onset AD. Rather, decreased Aβ clearance from the brain has been suggested [13]. In addition, there is accumulating evidence of blood-to-brain delivery of circulating Aβ, contributing to total parenchymal load [14]. Consistent with the concept of a vascular origin for cerebral Aβ was the finding that intravenous injection of anti-Aβ-IgG completely blocked the influx of peripheral Aβ across the BBB [13].

Plasma Aβ could be derived from vascular smooth muscle cells and endothelial cells, or from blood platelets which are an established and significant source of Aβ. However, in recent studies, we reported that the absorptive epithelial cells of the small intestine (enterocytes) also have substantial abundance of Aβ, secreted into blood associated with chylomicrons [15]. Our observations are the first evidence of significant tissue Aβ abundance in the absence of pathological amyloidosis. Interestingly, enterocytic Aβ was substantially increased with the ingestion of a diet enriched in saturated fat and cholesterol, but in contrast, was completely abolished by fasting. These findings may help to explain the mechanisms underlying epidemiological studies and animal feeding studies that have demonstrated a strong positive relationship between fat intake and accelerated amyloid pathology in AD [1–4,16–18]. We will describe in this article how dietary induced elevations in plasma Aβ could compromise BBB integrity, resulting in altered cerebral Aβ homeostasis and inflammatory sequelae. Our hypothesis is supported by studies in transgenic animal models developed to over-express Aβ particularly, but not exclusively in neurons. In these animals, a high-fat diet substantially exacerbates amyloid burden demonstrating that cerebrovascular deposition is influenced by circulatory effects. LaRue et al. [19] showed in one strain of mice (Tg2576), a greater than eightfold increase in peripheral delivery of Aβ across the BBB to the brain and in other studies by Giri et al. [20] it was found that exposure of brain endothelial cells to Aβ promotes migration of inflammatory cells.

2. Circulating amyloid-beta and blood–brain barrier integrity

Several studies have provided evidence of a vasoactive role of Aβ, with pathological manifestations prior to Aβ deposition. Aβ is vasoconstrictive and vessels treated with Aβ show significant endothelial cell damage with changes in the cell membrane, cytoplasm, nucleus and other organelles [21]. Soluble Aβ in contact with the cerebrovasculature may play a pathophysiological role, which accompanies or precedes Aβ deposition. Moreover, any such Aβ-induced cerebrovascular abnormalities could potentially result in cognitive impairment, for example, by contributing toward decreased cerebral perfusion.

Previous studies where Aβ was intravascularly administered involved only acute single injections and investigated transportation across, or sequestration within, brain capillaries [22–24]. Longer term administration of Aβ (2 weeks), resulted in a significantly compromised BBB and activated CNS glial cells [25]. Whilst these studies demonstrate regulatory responses following exogenous administration of Aβ, the physiological significance of these studies is presently unclear.

3. Chylomicron assimilation of amyloid-beta, blood and BBB kinetics

There is a growing body of evidence supporting the hypothesis that the BBB permeability is influenced by a variety of factors including humoral, endocrine and inflammatory mediators. However, the potential effect of diet on the BBB has to our knowledge not been studied. A number of centrally mediated changes in physiology and behaviour occur in response to diet. How these changes are mediated is not currently established. One way for diet to alter function in the central nervous system is through a disruption of the BBB, through the influence of circulating inflammatory cytokines, hormones or other mediators. Disruption of the BBB could allow inflammatory mediators or other circulating products to enter the brain parenchyma. However, to date BBB permeability has only been assessed in experimental colitis [26], or following acute intravenous injection of solubilised, exogenous Aβ [22–24].

The secretion, interaction with chaperone molecules, clearance and metabolism of Aβ are unclear. Koudinov et al. [27] found that Aβ in cerebrospinal fluid (CSF) was associated with a lipoprotein whose density was similar to plasma high-density-lipoproteins. Biere et al. [28] monitored the association of Aβ with plasma proteins in vitro and found that 5% bound to lipoproteins. Schwarzman and Goldgaber [29] incubated Aβ with CSF proteins and identified a peak of Aβ within very-dense-lipoproteins rich in precipitable transthyretin and Ghiso et al. [30] suggested that apolipoprotein (apo) J might be a primary vehicle of Aβ transport. Note however, that these studies using exogenous Aβ may be confounded because of the solubilising conditions used and because of the non-physiological introduction of Aβ into the brain or peripheral fluids.

We now present several lines of evidence suggesting that significant peripheral Aβ metabolism occurs in association

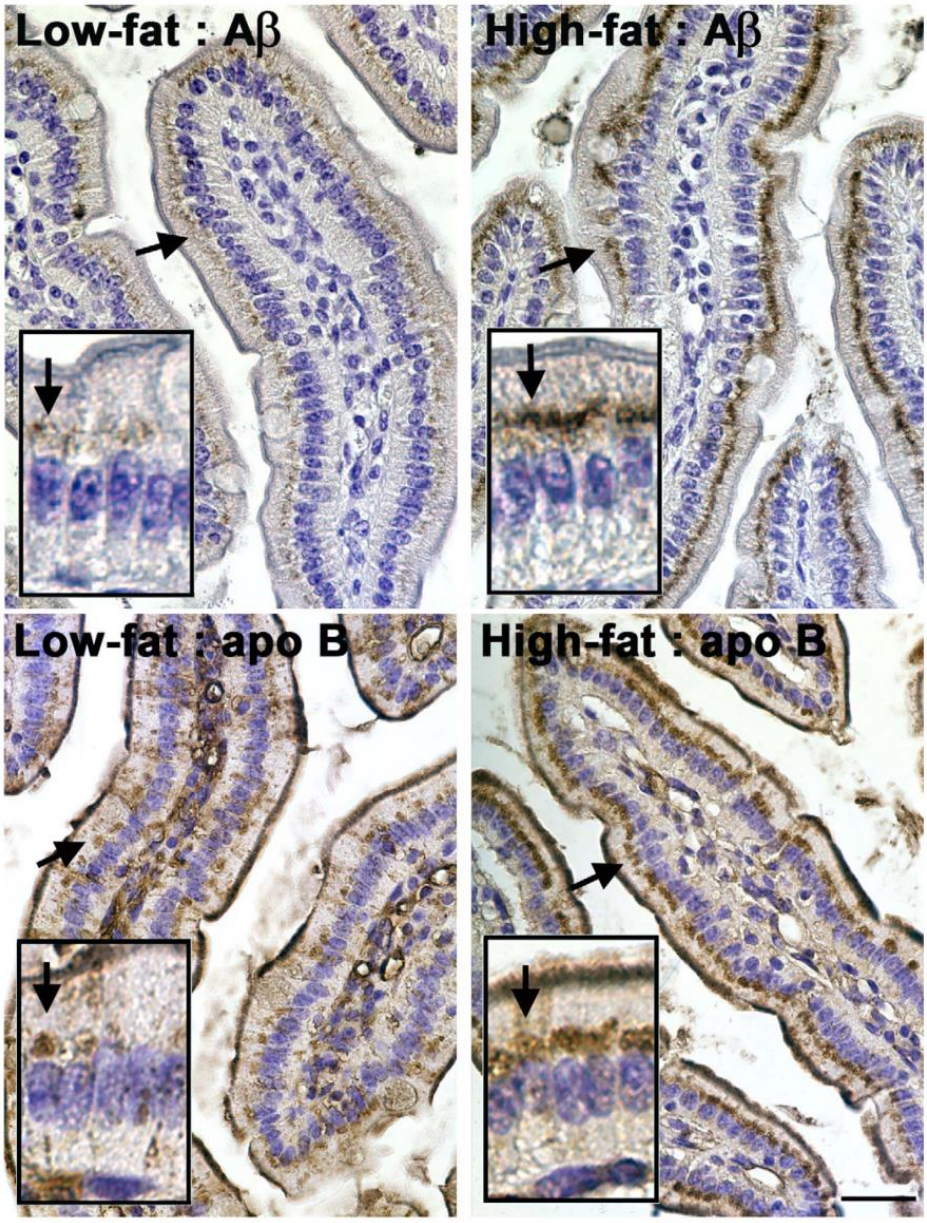


Fig. 1. Amyloid-beta (Aβ) is observed (by immunohistochemistry staining) within the perinuclear region of absorptive cells of the small intestine of wild-type mice maintained on a low-fat (4% (w/w) as poly-unsaturates) diet. Mice given saturated fats (16% (w/w)) and 0.5% cholesterol (w/w) had substantially greater abundance of amyloid-beta concomitant with increased apolipoprotein B48. Inset shows enterocytes at higher magnification. Bar indicates 20 μm.

with chylomicrons. In wild-type mice, there is abundance of Aβ in the epithelial (absorptive) cells of villi and in the crypts of the upper small intestine. Amyloid-beta expression is enhanced with saturated-fat feeding commensurate with an increase and apo B48, an obligatory component of nascent chylomicrons (Fig. 1). We found that a combined high saturated fat and cholesterol diet substantially enhanced enterocytic abundance of Aβ within the perinuclear region of the cell, consistent with exaggerated chylomicron biogenesis. A second line of evidence comes from clinical studies, where we found that following ingestion of a fatty meal, there is a transient increase in the plasma concentration of both the precursor protein and of Aβ, the latter in a triglyceride-rich plasma chylomicron fraction [31]. If Aβ, as our data suggest, is associated with chylomicrons, then a physiological function of Aβ may be to modulate the metabolism of dietary fats. Previous studies are consistent with this concept. Amyloid-beta attenuated hepatic uptake of chylomicron-like emulsions by 75%, promoting uptake by fat rich tissues including adipose and bone marrow [32].

Kinetic analysis in blood of doubly labelled chylomicron-Aβ showed that the majority of Aβ was cleared from plasma with uptake of the chylomicron particles, however, there was some delay in the clearance of Aβ relative to chylomicrons at later time points [32]. A divergence in kinetics of Aβ relative to the chylomicron particle is consistent with some apolipoprotein transfer and in the context of AD risk, this may be pathologically significant. In man the 'post-prandial' chylomicron excursion in response to a single standard meal, lasts for approximately 6–8 h. However, the transfer or shedding of Aβ from chylomicrons would suggest the possible genesis, or supplementation of a secondary pool of plasma Aβ with even longer plasma residency time. Given that humans are almost always in an absorptive state, the cumulative effect of high saturated fat diets on plasma Aβ homeostasis and thereafter BBB integrity may be significant.

4. Plasma lipoprotein amyloid-beta distribution

Clinically determining chylomicron-Aβ and indeed the plasma lipoprotein distribution of Aβ has proven to be difficult because hydrophobic lipids bind tightly to Aβ and mask antigenic epitopes [33]. Indeed, we found that delipidation leads to substantial loss of Aβ because of the tight binding of the protein with neutral lipids. Recently, we reported the distributional analysis of plasma lipoprotein-Aβ in normal subjects and those with probably AD [34]. For all subjects, we found the majority of plasma-Aβ to be associated with triglyceride-rich-lipoproteins (TRLs) inclusive of chylomicrons and their post-hydrolyzed remnants (lipoprotein fraction with $\rho \leq 1.019$ g/ml). For all lipoprotein groups (including LDL ($1.020 < \rho < 1.064$ g/ml) and HDL ($1.064 < \rho < 1.21$ g/ml)), $A\beta_{1-40}$ was the predominant isoform accounting for approximately 50% of the total and thereafter, equivalent amounts of the isoforms 1–42, 2–40, 1–38, 1–37 and 1–39 were found. Notably, the $A\beta_{1-37}$, $A\beta_{1-38}$ and $A\beta_{2-40}$ isoforms were significantly enriched within the TRL fraction of AD subjects and similar trends were observed for isoforms $A\beta_{1-39}$, $A\beta_{1-40}$ and $A\beta_{1-42}$. Interestingly, whilst AD subjects were normolipidemic, the concentration of apo B48 (an exclusive marker of chylomicrons) was significantly elevated in the post-absorptive state (17.4 ± 5.0 plasma vs 5.4 ± 1.1 μg/ml). Increased apo B48 is usually indicative of post-prandial dyslipidemia, an exaggerated but transient rise in plasma chylomicrons that occurs following the ingestion of dietary fats [35].

It is unclear how Aβ might become associated with chylomicrons or other chylomicron-apoprotein moieties. Amyloid-beta forms part of the amyloid multi-domain precursor protein and its predicted structure indicates that APP resembles a trans-membrane cell surface receptor. Amyloid-precursor-protein possesses a cysteine-rich motif not unlike several lipoprotein receptors that bind with very high affinity to apo E containing lipoproteins. Therefore, it is possible that chylomicrons assimilate endogenously produced Aβ during the proteolytic processing of APP in enterocytes. Once secreted into circulation, chylomicrons interact with lipases located on the surface of endothelial cells. As triglycerides are hydrolyzed, the chylomicrons accumulate apo E, which is known to bind Aβ. The association of chylomicrons with the plasma membrane and acquisition of apo E, may also contribute to Aβ acquisition. Furthermore, Aβ may exchange between chaperone macromolecules, not unlike the normal transfer of many apolipoproteins. However, none of these possibilities fully explain the significant intracellular abundance of Aβ found within the peri-nuclear vicinity of enterocytes. Rather, this and other studies which demonstrate Aβ within the rough endoplasmic reticulum (rER) and Golgi compartments, raise the possibility that Aβ associates directly with primordial lipoproteins during their biosynthesis. Indeed in cell culture exudates, Aβ is secreted exclusively as a lipoprotein complex [33].

Apolipoprotein B48, an obligatory structural component of chylomicrons requires successful 'lipidation,' specifically the addition of cholesteryl-ester for lipoprotein secretion to occur and at this point other apoproteins bind to the nascent lipoprotein. It is possible that intestinal secretion of Aβ might occur with the ingestion of cholesterol and other dietary fats.

5. Transgenic animal models of Alzheimer's disease

Indirect evidence for a role of TRLs in AD also comes from studies conducted in various strains of transgenic mice over-expressing the amyloid precursor protein (predominantly in neurons). In a recent study by Burgess et al. [36] plasma Aβ was substantially increased and this was positively related to plasma triglyceride concentration and with the onset of cerebrovascular and parenchymal amyloidosis. Moreover, plasma Aβ correlated with lipoprotein triglyceride secretion rates, possibly because of an overproduction phenomenon of lipoprotein-Aβ.

Consistent with the concept that circulating lipoprotein-Aβ may compromise BBB integrity and exacerbate amyloidosis, we now present preliminary evidence of plasma protein extravasation in APP/PS1 transgenic mice (Fig. 2). Significant immunoglobulin G (IgG) was observed in transgenic mice with enrichment associated with amyloid plaque. In contrast, wild-type mice only showed modest IgG staining in some blood vessels. Moreover, occludin expression (an endothelial tight junction protein) was substantially less in transgenic mice compared to wild-type controls (Fig. 2), consistent with diminished endothelial integrity.

6. Chylomicron amyloid-beta metabolism and apo E genotype in man

Considerable interest has focused on the putative relationship between Aβ kinetics and apo E, because of strong genetic evidence that links increased incidence of sporadic

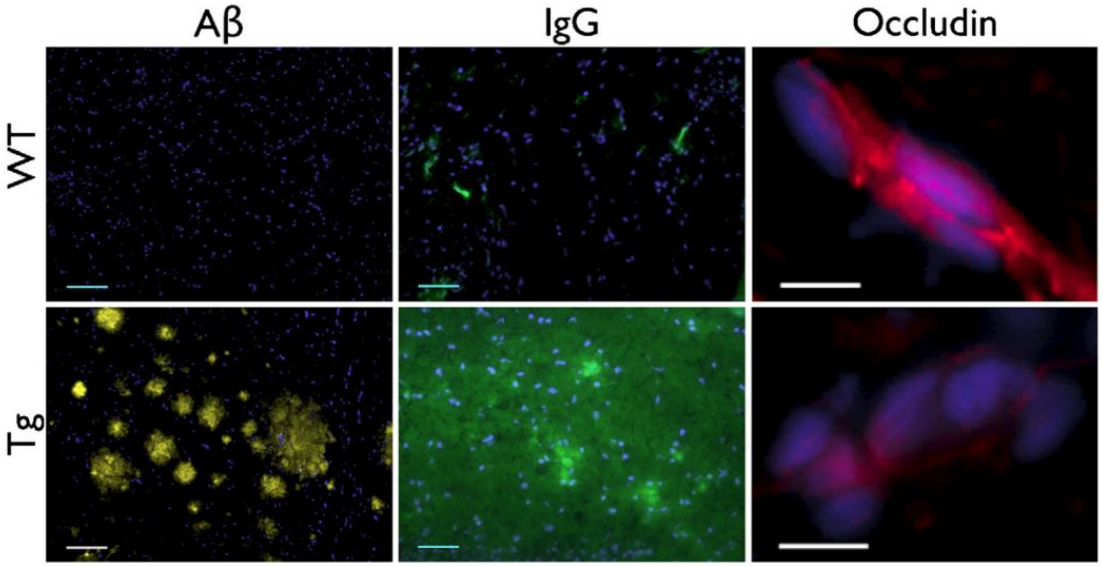


Fig. 2. Cerebral amyloid plaque is seen in a murine model of Alzheimer's disease (transgenic amyloid-precursor-protein/presenelin mice maintained on a high-fat diet), but not in wild-type controls. Concomitant with amyloidosis is widespread extravasation of immunoglobulin G, a surrogate marker of plasma proteins, with accentuated staining seen associated with amyloid deposits. Expression of occludin, an endothelial tight junction protein is attenuated in the transgenic mice compared to controls. Bars indicate 50 μm.

and familial late-onset AD in subjects with E4 isoforms [37] (of which there are three in man (apo-E4, -E3 and -E2)). Furthermore, apo E is found in dietary fat induced extracellular amyloid deposits, including vascular deposits. Greater than 90% of plasma apo E is associated with remnants of chylomicrons and hepatic very-low-density-lipoproteins (VLDL), that is, particles that have undergone triglyceride hydrolysis by endothelial lipases. However, interestingly there is a preferential distribution of apo E4 compared to apo E3 or apo E2 amongst remnant fractions containing greater amounts of residual triglyceride. Chylomicron remnants contain approximately twofold more triglyceride than hepatic VLDL-remnants and therefore presumably accumulate more apo E4 in subjects expressing this apolipoprotein isoform. Apo E4 may enhance Alzheimer's risk via several mechanisms, but we now introduce the concept that this may also be linked to aberrant chylomicron-Aβ metabolism. It has been proposed that lipidated and free apo E functions as a pathological chaperone of Aβ. LaDu [38] reported that apo E3 lipoproteins bound approximately 20 times more Aβ than apo E4 lipoproteins. If Aβ binding to chylomicrons is modulated by apo E, then based on the findings of LaDu, we would predict that in man, chylomicron remnants enriched in apo E4 will retain less Aβ, a phenomenon which may be detrimental to BBB integrity if the non-chylomicron bound form of the protein is more 'cytotoxic' than the lipoprotein bound form (Fig. 3).

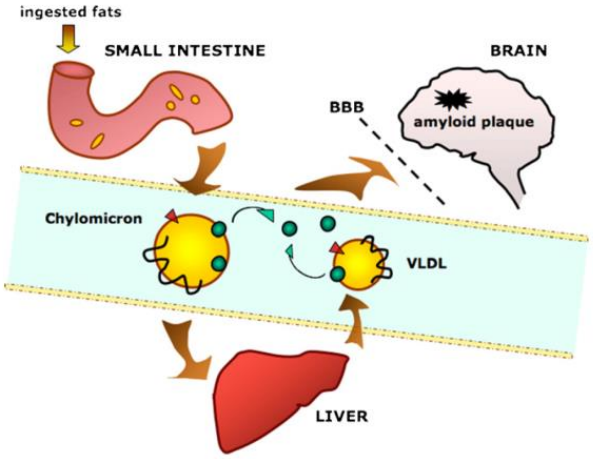


Fig. 3. Model for dietary induced modulation of blood–brain barrier integrity and amyloidosis. Saturated fats stimulate synthesis and secretion of chylomicron-associated amyloid-beta (Aβ). Dietary saturated fats may also increase hepatic secretion of very-low-density-lipoprotein (VLDL)-Aβ. Overproduction, coupled with diminished clearance by receptor pathways of remnant lipoproteins results in chronically elevated levels of plasma Aβ. Cerebrovascular integrity is compromised and there is increased blood-to-brain delivery of circulating Aβ.

7. Summary and conclusion

Population studies continue to support a role of dietary fats in Alzheimer's (AD). Laitinen et al. [39] reported that intake of unsaturated fats is protective, whereas intake of saturates increases risk of AD. In the Framingham study, the top quartile of plasma docosahexanoic acid (DHA) (profoundly influenced by diet) was associated with a 47% reduction in risk of all-cause dementia [40]. Irrefutable evidence continues to come from animal studies including by Oksman et

al. [41] who confirmed that saturates increased, whilst DHA decreased Aβ levels compared to soy oil diet. In cell culture studies, fatty acids increased presenilin 1, gamma secretase and Aβ independent of any increase in cellular cholesterol [42] and other evidence consistent with our hypothesis comes from Cullen et al. [43], who showed in human studies that Aβ deposits co-localized with blood proteins and microhemorrhages.

Elevated total cholesterol, LDL and triglyceride with normal HDL and TC/HDL ratio characterize the lipid profile in AD. However, MMSE (mini mental-state examination) does not correlate with lipid parameters suggesting no interaction between cholesterol and cognition in AD [44]. Inhibitors of HMG-CoA reductase (statins) reduce cardiovascular diseases and exhibit pleiotropic effects independent of lipid modification. Some of these effects may improve outcome or ameliorate symptoms of neurological disorders including AD, but the appropriateness of initiating statin therapy is not established at this time [45].

Recent evidence indicates that insulin resistance is associated with an increased relative risk for AD [46] and this notion was directly explored in Tg2576 mice which model AD-like neuropathology [47]. Ho et al. found that diet-induced insulin resistance promoted amyloidogenic Aβ. However, consistent with the hypothesis proposed in this article, the murine model would also have exaggerated plasma TRL-Aβ.

How dietary fats influence BBB function and the propensity for amyloidosis we contend would be useful in the context of AD prevention and quite possibly, in the development of nutrition-based intervention strategies. Collectively, accumulating evidence suggests that chronic consumption of foods rich in saturated fats may increase AD risk but the mechanisms for this are presently unknown. It is our contention that increased secretion and diminished clearance of lipoprotein associated Aβ in response to saturated fats leads compromised BBB integrity and a shift in Aβ kinetics from blood-to-brain. Evidence consistent with this hypothesis may provide novel nutrition and/or pharmacological strategies to reduce the prevalence or progression of Alzheimer's disease.

Conflicts of interest

None.

References

[1] Kalmijn S, Launer LJ, Ott A, et al. Dietary fat intake and the risk of incident dementia in the Rotterdam Study. Ann Rev 1997;42:776–82.
[2] Petot GJ, Friedland RP. Lipids, diet and Alzheimer's disease: an extended summary. J Neurol Sci 2004;226:31–3.
[3] Sparks DL, Scheff SW, Hunsacker 3rd JC, et al. Induction of Alzheimer-like beta-amyloid immunoreactivity in the brains of rabbits with dietary cholesterol. Exp Neurol 1994;126:88–94.
[4] Refolo LM, Malester B, LaFrancois J, et al. Hypercholesterolemia accelerates the Alzheimer's-like pathology in a transgenic mouse model. Neurobiol Dis 2000;4:321–31.
[5] Ellis RJ, Olichney JM, Thal LJ, et al. Cerebral amyloid angiopathy in the brains of patients with Alzheimer's disease. Neurology 1996;46:1592–6.
[6] Kalara RN. The blood–brain barrier and cerebral microcirculation in Alzheimer's disease. Cererbrovasc Brain Metab Rev 1992;4:226–60.
[7] Wisniewski HM, Vorbrodt J, Wegiel S. Amyloid angiopathy and blood–brain barrier changes in Alzheimer's disease. Ann NY Acad Sci 1997;826:161–72.
[8] Cullen KM. Perivascular astrocytes within Alzheimer's disease plaques. Neuroreport 1997;8:1961–6.
[9] Itagaki S, McGeer PL, Akiyama H, Shu S, Selkoe D. Relationship of microglia and astrocytes to amyloid deposits of Alzheimer disease. J Neuroimmunol 1989;24:173–82.
[10] De la Torre JC, Hachinski V. Cerbrovascular pathology in Alzheimer's disease. Ann N Y Acad Sci 1997;826:523.
[11] Gandy S. The role of cerebral amyloid beta accumulation in common forms of Alzheimer's disease. J Clin Invest 2005;115:1121–9.
[12] Joachim CL, Duffy LK, Morris JH, Selko DJ. Protein chemical and immunocytochemical studies of meningovascular beta-amyloid protein in Alzheimer's disease and normal aging. Brain Res 1988;474:100–11.
[13] Deane R, Sagare A, Hamm K, et al. IgG-assisted age-dependent clearance of Alzeheimer's amyloid beta peptide by the blood–brain barrier neonatal Fc receptor. J Neurosci 2005;25:11495–503.
[14] Mackic JB, Bading J, Ghiso J, et al. Circulating amyloid-beta peptide crosses the blood–brain barrier in aged monkeys and contributes to Alzheimer's disease lesions. Vascul Pharmacol 2002;38:308–13.
[15] Galloway S, Jian L, Chew S, Mamo JCL. Beta-amyloid or its precursor protein is found in epithelial cells of the small intestine and is stimulated by high fat feeding. J Nutr Biochem 2007;18:279–84.
[16] Shie FS, Jin LW, Cook DG, Leverenz JB, LeBoeuf RC. Diet-induced hypercholesterolemia enhances brain Abeta accumulation in transgenic mice. Neuroreport 2002;13:455–9.
[17] Levin-Allerhand JA, Lominska CE, Smith JD. Increased amyloid levels in APPSWE transgenic mice treated chronically with a physiological high-fat cholesterol diet. J Nutr Health Ageing 2002;6:315–9.
[18] George AJ, Holsinger RM, McClean CA, et al. APP intracellular domain is increased and soluble Abeta is reduced with diet-induced hypercholesterolemia in a transgenic mouse model of Alzheimer's disease. Neurobiol Dis 2004;16:124–32.
[19] LaRue B, Hogg E, Sagare A, et al. Method for measurement of the blood–brain barrier permeability in the perfused mouse brain. J Neurosci Methods 2004;138:233–42.
[20] Giri R, Selvaraj S, Miller CA, et al. Effect of endothelial cell polarity on beta-amyloid-induced migration of monocytes across normal and AD endothelium. Am J Physiol Cell Physiol 2002;283:C895–904.
[21] Thomas T, McLendon ET, Sutton G, et al. Cerebrovascular endothelial dysfunction mediated by beta-amyloid. Neuroreport 1997;8:1387–91.
[22] Mannes LM, Banks WA, Podlisny MB, et al. Passage of human amyloid beta protein across the murine blood–brain barrier. Life Sci 1994;55:1643–50.
[23] Martel CL, Mackic JB, McComb J, et al. Blood–brain barrier uptake of the 40 and 42 amino acid sequences of circulating Alzheimer's amyloid beta in guinea pigs. Neurosci Lett 1996;206:157–60.
[24] Zlokovic BV, Ghiso J, Mackie JB, et al. Blood–brain barrier transport of circulating Alzhimer's amyloid beta. Biochem Biophys Res Commun 1993;197:1034–40.
[25] George CS, Arendash GW, Kalaria RN, et al. Intravascular infusions of soluble beta-amyloid compromise the blood–brain barrier, activate CNS glial cells and induce peripheral hemorrhage. Brain Res 1999;818:105–17.
[26] Natah SS, Mouihate A, Pittman QJ, et al. Disruption of the blood–brain barrier during TNBS colitis. Neurogastroenterol Motil 2005;17:433–46.

[27] Koudinov A, Matsubara E, Frangione B, Ghiso J. Biochem Biophys Res Commun 1994;205:1163–71.
[28] Biere AL, Ostaszewski B, Stimson ER, et al. Amyloid β-peptide is transported on lipoproteins and albumin in human plasma. J Biol Chem 1996;271:32916–22.
[29] Schwarzman AL, Goldgaber D. Interaction of transthyretin with amyloid β-protein: binding and inhibition of amyloid formation. Ciba Found Symp 1996;199:146–64.
[30] Ghiso J, Matsubara E, Koudinov A, et al. The cerebrospinal-fluid soluble form of Alzheimer's amyloid beta is complexed to SP-40,40 (apolipoprotein J), an inhibitor of the complement membrane-attack complex. Biochem J 1993;293:27–30.
[31] Boyt AA, Taddei K, Hallmayer J, et al. Relationship between lipid metabolism and plasma concentration of amyloid precursor protein and apolipoprotein E. Alzheimer's Rep 1999;2:339–46.
[32] James AP, Pal S, Gennat HC, Vine DF, Mamo JC. The incorporation and metabolism of amyloid-beta into chylomicron-like lipid emulsions. J Alzheimer's Dis 2003;5:179–88.
[33] James AP, Mamo JC. The immunodetection of lipoprotein bound amyloid-β is attenuated because of the presence of lipids. Ann Clin Biochem 2005;42:70–2.
[34] Mamo JCL, Jian L, Flicker L, Esselman H, Wiltfgang J. Plasma lipoprotein-beta-amyloid in subjects with Alzheimer's disease or mild cognitive impairment. Ann Clin Biochem 2008;45:395–403.
[35] Smith D, Watts GF, Dane-Stewart C, Mamo JCL. The postprandial chylomicron response can be predicted by a single measurement of plasma apolipoprotein B48 in the fasting state. Eur J Clin Invest 1999;29:204–9.
[36] Burgess BL, McIsaac SA, Naus KE, et al. Elevated triglyceride levels precede amyloid deposition in Alzheimer's disease models with efficient brain to blood transport of Aβ. Neurobiol Dis 2006;24:114–27.
[37] Poirier J, Davignon J, Bouthillier D, et al. Apolipoprotein E polymporphism and Alzheimer's disease. Lancet 1993;342:697–9.
[38] LaDu MJ, Lukens JR, Reardon CA, Getz GS. Association of human and rabbit apolipoprotein E with β-amyloid. J Neurosci Res 1997;49:9–18.
[39] Laitinen MH, NNgandu T, Rovio S, et al. Fat intake at midlife and risk of dementia and Alzheimer's disease: a population based study. Dement Geriatr Cogn Disord 2006;22:99–107.
[40] Schaefer EJ, Bongard V, Beiser AS, et al. Plasma phosphatidylcholine docosahexanoic acid content and risk of dementia and Alzhimer disease: the Framingham Heart Study. Arch Neurol 2006;63:1545–50.
[41] Oksman M, Iivonen H, Hogyes E, et al. Impact of different saturated fatty acid, polyunsaturated fatty acid and cholesterol containing diets on beta-amyloid accumulation in APP/PS1 transgenic mice. Neurobiol Dis 2006;23:563–72.
[42] Liu Y, Yang L, Conde-Knape K, et al. Fatty acids increase presenilin-1 levels and gamma-secretase activity in PSwt-1 cells. J Lipid Res 2004;45:2368–76.
[43] Cullen KM, Kocsi Z, Stone J. Microvascular pathology in the ageing human brain: evidence that senile plaques are sites of microhaemorrhages. Neurobiol Aging 2006;27:1786–96.
[44] Sabbagh M, Zahiri HR, Ceimo J, et al. Is there a characteristic lipid profile in Alzheimer's disease? J Alzheimer's Dis 2004;6:585–9.
[45] Reiss AB, Wirkowski E. Role of HMG CoA reductase inhibitors in neurological disorders: progress to date. Drugs 2007;67:2111–20.
[46] Razay G, Vreugdenhil A, Wilcock G. The metabolic syndrome and Alzheimer's disease. Arch Neurol 2007;64:93–6.
[47] Ho L, Qin W, Pompl PN, et al. Diet-induced insulin resistance promotes amyloidosis in a transgenic mouse model of Alzheimer's disease. FASEB J 2004;18:902–4.

Article 2:

Pallebage-Gamarallage, M. M., **S. Galloway**, R. Johnsen, L. Jian, S. Dhaliwal, and J. C. Mamo. "The Effect of Exogenous Cholesterol and Lipid-Modulating Agents on Enterocytic Amyloid-Beta Abundance." [In eng]. *Br J Nutr* 101, no. 3 (Feb 2009): 340-7.

Synopsis:

The manuscript titled "The Effect of Exogenous Cholesterol and Lipid-Modulating Agents on Enterocytic Amyloid-Beta Abundance" is complementary to my PhD candidacy by directly supporting my objectives. This research article investigates the regulation of enterocyte Aβ by cholesterol and cholesterol modulating agents and considers the role of statins as a therapeutic intervention to counter balance dietary induced enterocyte Aβ. Results presented here confirm that endogenous or exogenous (dietary) cholesterol plays a role in addition to dietary fat in relation to Aβ abundance. Saturated fat feeding was used as a positive control and expression of enterocytic Aβ was significantly greater than cholesterol fed diet, indicating that the SFA component of the diet alone was sufficient enough to obtain a response. I contributed to this study by appraisal of manuscript and by assisting in animal care and tissue collection.

Background:

Several studies in animals show cholesterol feeding promotes deposition of Aβ in brain. In rabbits fed 2 % (w/w) cholesterol, there was an increase in neuronal accumulation of Aβ which was increased with feeding time (Sparks et al. 1994). Interestingly, removal of cholesterol content from the diet reversed the observation (Sparks 1996). Similarly, dietary cholesterol increased cerebral amyloid plaques in transgenic APPsw and PS1 mice (Refolo et al. 2000). Intracellular (neuronal) abundance of Aβ correlated positively with increased cholesterol (Sparks et al. 1994). Consistent with this notion, statin treatment induced reduction of intracellular cholesterol reduced Aβ (Fassbender et al. 2001, Wolozin 2004). Ezetimibe selectively inhibits cholesterol absorption (Davis et al. 2004) and Atorvastatin

(ATOR) inhibits endogenous cholesterol biogenesis by inhibiting 3-hydroxyl-3-methylglutaryl CoA reductase inhibitor (Naoumova et al. 1997). Both ezetimibe and atorvastatin were incorporated separately into low-fat 2 % (w/w) cholesterol feed to determine effects on enterocytic Aβ.

Methods:

Wild-type C57BL/6J mice were place on either low-fat and cholesterol-free diet (LF) or low-fat and 2 % cholesterol diet (HC). Another group fed a saturated fat diet was used as a comparison. Both LF and HC diets incorporated ezetimibe or atorvastatin within the diet making 6 groups of animals in total (4 statin + 2 control groups). Samples of the small intestine were collected for immunohistochemistry analysis and results were graded via semi-quantitative methods according to previous studies.

Results:

Enterocytic Aβ was found within the perinuclear location of enterocytes, the site of Golgi and rER. In comparison to LF feeding, HC diet significantly ($P < 0.000$) decreased enterocytic abundance of Aβ. Inhibition of cholesterol absorption by ezetimibe in LF group reversed the suppressive effects ($P < 0.000$) of cholesterol whilst atorvastatin in LF group enhanced enterocytic Aβ ($P = 0.016$). Results obtained from HC + EZE was comparative to LF alone, implying EZE treatment did not have other unknown effects on enterocytic Aβ. Furthermore the increased enterocytic Aβ was observed in LF group not HC; the latter group displayed Aβ abundance not different from HC + ATOR indicating that ATOR did not have pleiotropic effects on enterocyte Aβ. Saturated fat feeding increased enterocytic Aβ within the perinuclear region compared to LF control group.

Discussion:

The presence of Aβ within enterocytes and attenuating effects of cholesterol inhibiting agents can translate to changes in the balance of production and secretion of Aβ. Plasma cholesterol was not significantly different between LF, HC diets and EZE, ATOR groups indicating that secretion of enterocyte Aβ-lipoproteins was likely

to be unchanged. Rather, modulation of enterocytic Aβ by cholesterol and statins could affect synthesis of Aβ with lipoproteins. Findings by Park et al. (2003) support the results of this study, and show that cholesterol inhibition by lovastatin increased production of Aβ in transgenic AD mice. Studies indicate that intracellular presence of both exogenous (absorbed) and endogenous cholesterol is suppressive towards enterocytic Aβ abundance, possibly via production pathways. Beta-amyloid homeostasis can be regulated by distribution of membrane non-esterified cholesterol and intracellular cholesteryl esters (Abad-Rodriguez et al. 2004). Cholesterol trafficking in neurons lowered β-secretase and increased γ-secretase which increased intracellular Aβ (Runz et al. 2002). These cholesterol esters are also implicated in genesis and secretion of chylomicrons (Shen et al. 2001, Simons and Ikonen 2000). The present study was not designed to assess the risk of intestinal Aβ and its modulation by cholesterol towards development of AD. However, this study suggests that modulation of enterocyte Aβ production and secretion may reduce risk of developing AD via downstream events involving transport of enterocytic Aβ-lipoproteins across the BBB.

British Journal of Nutrition (2009), **101**, 340–347
doi:10.1017/S0007114508012269

The effect of exogenous cholesterol and lipid-modulating agents on enterocytic amyloid-β abundance

Menuka M. Pallebage-Gamarallage[1,2,3], Susan Galloway[2,3], Russell Johnsen[4,5], Le Jian[2,3], Satvinder Dhaliwal[2,3] and John C. L. Mamo[2,3]*

[1]*School of Biomedical Sciences, Curtin University of Technology, Perth, WA, Australia*
[2]*School of Public Health, Curtin University of Technology, Perth, WA, Australia*
[3]*Australian Technology Network, Centre for Metabolic Fitness, Perth, WA, Australia*
[4]*Center for Neuromuscular and Neurological Disorders, University of Western Australia, WA, Australia*
[5]*The Australian Neuromuscular Research Institute, Nedlands, WA, Australia*

(Received 12 December 2007 – Revised 23 April 2008 – Accepted 29 April 2008 – First published online 17 July 2008)

Dietary cholesterol may influence Alzheimer's disease risk, because it regulates the synthesis of amyloid-β. It was recently demonstrated in enterocytes of wild-type mice that intracellular amyloid-β expression is enhanced in response to a high-fat diet made up of SFA and cholesterol. Intestinally derived amyloid-β may be associated with postprandial lipoproteins in response to dietary fats and could be a key regulator in chylomicron metabolism. The present study was designed to investigate the role of cholesterol in modulating amyloid-β abundance in enterocytes. Wild-type mice were fed a low-fat diet supplemented with 2 % (w/w) cholesterol. The effects of cholesterol absorption inhibition and cholesterol biosynthesis inhibition utilising ezetimibe and atorvastatin, respectively, were also studied. Quantitative immunohistochemistry was utilised to determine enterocytic amyloid-β homeostasis. We found that enterocytic amyloid-β concentration was significantly attenuated in mice fed the 2 % (w/w) cholesterol diet. However, blocking cholesterol absorption reversed the cholesterol-feeding effect. Consistent with a suppressive effect of cholesterol on enterocytic amyloid-β abundance, atorvastatin, an inhibitor of cholesterol biosynthesis, enhanced amyloid-β. However, providing exogenous cholesterol abolished the atorvastatin-induced effect. In contrast to the suppression of enterocytic amyloid-β by dietary cholesterol, mice fed a diet enriched in SFA had markedly greater abundance. Collectively, the findings suggest that exogenous and endogenous cholesterol reduce amyloid-β concentration in enterocytes by suppressing production, or enhancing secretion associated with postprandial lipoproteins. Intestinally derived amyloid-β will contribute to the pool of plasma protein and may influence cerebral amyloid homeostasis by altering the bi-directional transfer across the blood–brain barrier.

Amyloid-β: Cholesterol: Chylomicrons: Cholesterol-modulating drugs

Amyloid-β (Aβ) is the main component of proteinaceous deposits found in the brain tissue of subjects with Alzheimer's disease[1]. Aβ is a polypeptide of thirty-nine to forty-three amino acids produced from proteolytic cleavage of the Aβ protein precursor (AβPP)[2,3] by sequential action of β- and γ-secretases[3,4]. Cleavage of AβPP within the Aβ domain at amino acid 17 by the α-secretase pathway will alternatively generate a membrane-bound carboxyl-terminal derivative, which is non-pathogenic[3]. Historically, Aβ generation was thought to occur only at the cell membrane[5,6]. However, cell-culture studies have shown that Aβ is also generated at the endoplasmic reticulum and secreted *via* the Golgi apparatus[7–9].

The origin of cerebrovascular Aβ deposits is controversial. There is little evidence for increased Aβ production in sporadic, late-onset Alzheimer's disease. Rather, decreased Aβ clearance from the brain has been put forward as one alternative hypothesis[10]. A number of studies have also shown *in vivo* transport of circulating Aβ across the blood–brain barrier, thereby contributing to total brain parenchymal Aβ load[11]. Consistent with the concept of a vascular origin for cerebral Aβ was the finding that intravenous injection of anti-Aβ-IgG completely blocked the influx of peripheral Aβ across the blood–brain barrier[10]. Circulatory Aβ could be derived from vascular smooth muscle cells and endothelial cells[6,12], or from blood platelets[13]. However, in recent studies, we also reported that the absorptive epithelial cells of the small intestine have substantial abundance of Aβ[14].

We found that enterocytic Aβ was substantially increased with the ingestion of a diet enriched in saturated fat and cholesterol, but, in contrast, was completely abolished by fasting[14], clearly showing dietary regulation. These findings may provide insight into the mechanisms underlying epidemiological studies and animal feeding studies that have demonstrated a positive relationship between fat intakes and accelerated amyloid pathology in Alzheimer's disease[15–18]. It is our contention that dietary fat-induced elevations in plasma Aβ could

Abbreviations: Aβ, amyloid-β; AβPP, amyloid-β protein precursor; HC, high-cholesterol; LF, low-fat.

* **Corresponding author:** Professor John Mamo, fax +61 8 92662958, email J.Mamo@Curtin.edu.au

BJN British Journal of Nutrition

compromise blood–brain barrier integrity, resulting in altered cerebral Aβ homeostasis and inflammatory sequelae. Our hypothesis is supported by studies in transgenic animal models that over-express the AβPP in neurons[19,20]. In these animals, a high-fat diet exacerbates Aβ burden, demonstrating that cerebrovascular deposition is influenced by circulatory effects, irrespective of the actual cellular origin of the Aβ peptide.

Several animal studies suggest that cholesterol is a pro-amyloidogenic dietary lipid. Rabbits fed 2 % (w/w) cholesterol have a dramatic increase in intraneuronal Aβ accumulation positively associated with the duration of feeding[18] and this is reversed when exogenous cholesterol is removed from the diet[21]. Similarly, in young double-transgenic APPsw and $PS1_{M146V}$ mice, dietary cholesterol significantly accelerates Aβ deposition[16]. In neuronal cells, Aβ production was positively associated with cholesterol availability[18] and, conversely, treatment with cholesterol synthesis or cholesterol esterification inhibitors negatively modulated Aβ biogenesis[22,23].

Enterocytes at the proximal region of the small intestine are responsible for the absorption of dietary cholesterol, released into the lymphatics primarily as esters associated with chylomicrons[24,25]. A number of studies have demonstrated that cholesterol regulates chylomicron biosynthesis[26–28]; hence, our previous finding of enhanced Aβ abundance in enterocytes in response to a high-fat dietary regimen could therefore reflect a cholesterol-induced stimulation of Aβ production. Indeed, several lines of evidence suggest that intestinally derived Aβ forms part of the chylomicron structure and thereafter serves as a regulatory apolipoprotein[29,30].

In the present study we compared wild-type mice that were maintained on sterol-free *v.* cholesterol-supplemented feed. An additional group of cholesterol-fed mice was also provided with ezetimibe, a potent compound of the 2-azitidinone class of drugs[31] that inhibits cholesterol absorption[32]. Control mice, fed a sterol-free diet, given ezetimibe alone, were used to rule out pleiotropic effects of the agent.

The effects of cholesterol biosynthesis inhibition on enterocytic Aβ abundance was also studied in mice that were given atorvastatin, a potent 3-hydroxy-3-methylglutaryl CoA reductase inhibitor (the rate-limiting step of the cholesterol biosynthetic pathway)[33]. Thereafter, we investigated whether the purported effect of statin therapy would be overcome by provision of dietary cholesterol supplementation.

Methods and materials

Animals and diet conditions

The animal housing, handling and experimental procedures described for the present study were approved by the Curtin University Animal Experimentation and Ethics Committee. Female wild-type mice (C57BL/6J), aged 6 weeks, were housed in groups and randomly divided into the diet and drug treatment groups (six mice per group). All mice were maintained in a 12 h light and dark cycle room, at 22°C and with free access to water and food. The control low-fat (LF) cholesterol-free group of mice was fed a semi-purified diet (AIN-93M; Glen Forrest Stockfeeds, Perth, Western Australia) containing 4 % (w/w) as total fat. Cholesterol was incorporated at 2 % (w/w) into the chow pellets in the sterol-supplemented group (SF06-056; Glen Forrest Stockfeeds). Mice treated with ezetimibe (Ezetrol; Schering-Plough Pty Limited, Baulkham Hill, NSW, Australia) also had the drug incorporated into chow at 12 mg/kg food and atorvastatin (Lipitor; Pfizer, West Ryde, NSW, Australia) was included at a dose of 20 mg/kg at the time of feed manufacture.

Tissue collection and sample preparation

The mice were fed with their respective diets for a period of 4 weeks and were weighed weekly. At the end of the intervention period, mice were anaesthetised with phenobarbitone (45 mg/kg intraperitoneally) and exsanguinated by cardiac puncture. Blood was collected into EDTA tubes and stored in ice. Plasma was separated by short speed centrifugation at 4°C and stored at −80°C.

The small intestine was isolated and flushed with chilled PBS (pH 7·4). A 2 cm segment of the small intestine distal to the duodenum was fixed in 10 % buffered formal saline for a minimum of 24 h, processed and longitudinal segments embedded in paraffin wax. Serial sections of 5 μm thick were cut on a microtome and mounted on silanised slides for histology and immunohistochemistry.

Immunohistochemistry

Intestinal tissue sections (5 μm) were deparaffinised, rehydrated and immunohistochemistry analysis was done as previously described[14]. Briefly, the sections were exposed to 3 % H_2O_2 in methanol for 30 min to quench endogenous peroxidase activity, washed and incubated in blocking serum (20 % goat serum) before overnight incubation at 4°C with polyclonal rabbit anti-human $A\beta_{1-40/42}$ antiserum (AB5076; Chemicon, Temecula, CA, USA), diluted to 1:1000 with 10 % goat serum. We previously established specificity by replacing the primary antibody with an irrelevant serum or with PBS and by competition immunohistochemistry analysis. For the latter, the primary antisera were pre-mixed with solubilised Aβ. Cerebral tissues from transgenic mice (Tg2576sw) expressing familial human APP_{695} with established plaques were used as positive controls. Slides were washed in PBS and incubated with biotinylated goat anti-rabbit secondary antibody (1:1000 dilution) (E 0432; Dako, Carpinteria, CA, USA), followed by avidin–biotin–peroxidase complex (ABC/HRP) (K 0377; Dako) for 45 min at room temperature. Positive immunostaining was established with liquid diaminobenzidine plus (DAB +) substrate chromogen kit (K 3467; Dako). Sections were then counterstained with Harris's haematoxylin.

Quantitative immunohistochemical analysis

The intensity of immunostaining for Aβ was quantified as previously described[14,34]. Stained sections were observed with an AxioVert 200M microscope (Zeiss, Jena, Germany). Six mice per group were investigated with duplicate tissue blocks prepared for each group. The absorptive epithelial cells of the small intestine were assessed by a blinded investigator from twenty randomly selected villi per intestine, and at least 100 cells in each villus were counted. The intensity of Aβ immunostaining was graded as negative (0), mild

(+1), moderate (+2) and intense (+3) at ×200 magnification. The number of cells with different staining intensity was counted for each villus.

Imaging

Digital images for photomicroscopy were acquired by an AxioCam HRc camera (Zeiss, Jena, Germany). Images were captured under identical settings utilising AxioVision software (version 4.5).

Cholesterol and triacylglycerol analysis

Plasma cholesterol and TAG were determined in duplicate by enzymic assays (Randox Laboratories Ltd, Crumlin, Co. Antrim, UK) and according to the manufacturer's instructions.

Statistical analysis

Statistical analysis of correlation between the intensity of Aβ staining and the feeding groups was determined by the χ^2 test. Plasma lipid data were analysed by ANOVA to assess the main effects of dietary cholesterol, cholesterol absorption inhibition (by ezetimibe) and cholesterol biosynthesis inhibition (by atorvastatin) and their two-way interactions. *Post hoc* comparison of means was done if the associated main effect or interaction was statistically significant within the ANOVA procedure. P values $<0{\cdot}05$ were considered to be statistically significant.

Results

Body weight and plasma cholesterol and triacylglycerol levels

The body weights post-dietary and drug intervention and plasma lipids are given in Table 1. The diet and drug interventions were well tolerated. Weight gain was similar for all groups of mice; however, the final body weight of LF mice given atorvastatin was modestly less than the control LF group ($P=0{\cdot}026$). Furthermore, LF mice given atorvastatin and ezetimibe also had lower final body weights when compared with the cholesterol-supplemented group (high-cholesterol; HC). Plasma lipids were not increased in response to dietary cholesterol supplementation, nor significantly influenced by either ezetimibe or atorvastatin. However, the TAG concentration for the HC + atorvastatin group was slightly lower than the control LF group ($P=0{\cdot}01$).

The pattern of amyloid-β distribution in absorptive epithelial cells of the small intestine of mice

Staining for Aβ in the small intestine is shown in Fig. 1. Positive Aβ staining was observed in absorptive epithelial cells for all groups of mice. Aβ immunostaining was found throughout the villi, increasing with proximity to the intestinal lumen. The Aβ was enriched within the perinuclear region of the enterocytes consistent with the sites of the Golgi apparatus and the rough endoplasmic reticulum, and the overall pattern of Aβ distribution between treatments was similar. A decreasing gradient of Aβ staining was evident from the perinuclear region through the cytoplasm and lacteals.

Table 1. Effect of various feeding regimens on average weight, and serum cholesterol and TAG levels in wild-type mice (C57BL/6J)*

(Mean values with their standard errors for six mice per group)

	Weight (g)		Total serum cholesterol (mM)		Serum TAG (mM)	
Feeding regimen	Mean	SEM	Mean	SEM	Mean	SEM
LF (df 5)	19·20	0·60	2·50	0·16	0·87	0·12
HC (df 5)	20·53	0·67	2·43	0·12	0·53	0·04
LF + atorvastatin (df 5)	18·91†‡	0·20	2·25	0·05	0·55	0·02
LF + ezetimibe (df 5)	19·10‡	0·24	1·94	0·29	0·61	0·13
HC + atorvastatin (df 5)	19·98	0·34	2·70	0·26	0·50†	0·07
HC + ezetimibe (df 5)	20·27	0·57	1·95	0·21	0·72	0·07

LF, low-fat; HC, high-cholesterol.

* Wild-type mice (C57BL/6J) were randomised to the six different feeding regimens and were fed their respective diets for 4 weeks. Weights and total serum cholesterol and TAG levels at the end of the experiment were compared between the groups with *post hoc* comparison of means within the ANOVA procedure. The F statistic for weight between all groups is 10·044; for total serum cholesterol, F 2·458; for serum TAG, F 2·843.

† Mean value was significantly lower than that of the LF group ($P<0{\cdot}05$).

‡ Mean value was significantly lower than that of the HC group ($P<0{\cdot}05$).

The effect of dietary cholesterol and the cholesterol absorption inhibitor ezetimibe on amyloid-β abundance in small-intestinal enterocytes

Enterocytic Aβ determined in mice given a sterol-free diet (LF) or a diet containing 2 % cholesterol is shown as Figs. 1 (A) and (B), respectively, and quantitative analysis for the intensity of Aβ immunostaining is indicated in Fig. 2. Cholesterol-supplemented mice showed a significant reduction in Aβ staining in the perinuclear regions of the absorptive enterocytes in comparison with the LF group ($P<0{\cdot}0001$; Pearson's χ^2 85·206; df 3) and indeed a larger percentage of absorptive epithelial cells had no discernible staining (Fig. 2). The control group had a greater proportion of cells with mild and moderate staining (Fig. 2). In contrast, the majority of absorptive epithelial cells in the cholesterol-fed group showed no Aβ staining.

The inhibition of enterocytic Aβ staining as a consequence of dietary cholesterol supplementation could be reversed by co-treatment with ezetimibe (Fig. 1 (C)). The intensity of perinuclear Aβ expression was enhanced in comparison with the HC group ($P<0{\cdot}0001$; Pearson's χ^2 80·215; df 3) but was not significantly different from the control LF group. Essentially all cells showed mild–moderate staining intensity and the pattern of distribution was unchanged (Fig. 2). To exclude pleiotropic effects independent of cholesterol absorption inhibition, mice were given ezetimibe in the absence of dietary cholesterol supplementation. No significant difference in intensity or distribution of staining was seen when compared with the LF control group (Fig. 1 (F)).

The effect of cholesterol biosynthesis inhibition by atorvastatin and cholesterol supplementation on amyloid-β abundance in small-intestinal enterocytes

To explore whether endogenous cholesterol biosynthesis regulates Aβ abundance in enterocytes, control LF-fed mice were

NS British Journal of Nutrition

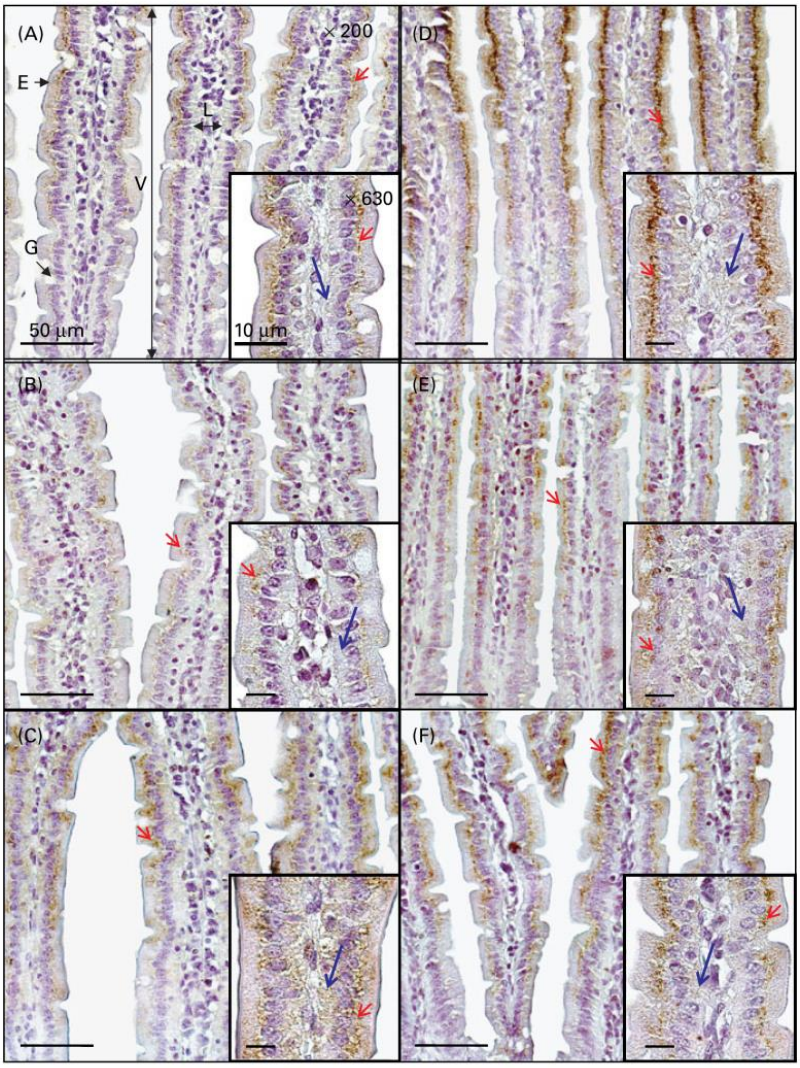


Fig. 1. Amyloid-β (Aβ) expression in enterocytes of wild-type mice fed low-fat (LF) (A), high-cholesterol (2 %; HC) (B), HC + ezetimibe (C), LF + atorvastatin (D), HC + atorvastatin (E) and LF + ezetimibe (F) dietary regimens for 4 weeks. Intestinal sections showing villi at increasing magnification show the pattern of Aβ distribution in the absorptive epithelial cells of the small intestine. A significant amount of Aβ (red arrow) was found concentrated within the perinuclear region of the absorptive enterocytes. Aβ staining was also visible in the lacteals (blue arrow). Aβ concentration was attenuated in HC-fed mice when compared with the LF mouse group, free of drug treatment (see also Fig. 2). The intensity of Aβ staining was enhanced in the HC + ezetimibe group compared with the HC group. Furthermore, atorvastatin increased Aβ immunostaining in the LF group. Large frames: magnification ×200; scale bar 50 μm. Inset frames: magnification ×630; scale bar 10 μm. E, enterocyte; G, goblet cell; L, lacteal; V, villus.

treated with atorvastatin (Fig. 1 (D)). We found a pronounced increase in staining intensity as a consequence of atorvastatin treatment, with approximately 20 % of enterocytes having intense colouration and the remainder with moderate to mild staining ($P=0{\cdot}016$; Pearson's χ^2 7·409; df 3) (Fig. 2). To indirectly explore if the atorvastatin-induced effect in Aβ staining was as a consequence of decreased cellular cholesterol abundance, another group of mice was given atorvastatin concomitant with dietary cholesterol. Exogenous cholesterol was found to significantly attenuate but not completely normalise the atorvastatin-mediated effect (Fig. 1 (E)). A large proportion of cells showed no Aβ staining (60 %), with essentially the remainder being classified as mild intensity (Fig. 2).

The effect of saturated fat feeding on amyloid-β abundance in small-intestinal enterocytes

To explore whether it was the SFA component of the diet which induced enterocytic Aβ accumulation previously reported[14], another group of mice were fed sterol-free chow supplemented with 20 % saturated fats. Figure 3 shows substantially exaggerated Aβ in the enterocytes of the mice given saturates.

Discussion

We reported that wild-type mice given a diet enriched in saturated fat and cholesterol had substantially greater

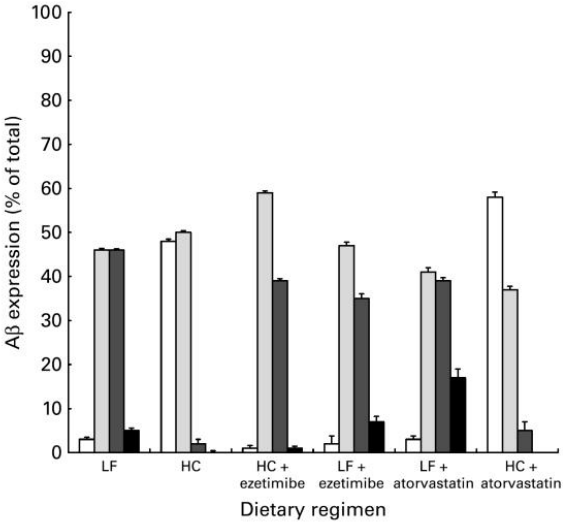


Fig. 2. Semi-quantitative analysis of enterocytic amyloid-β (Aβ) expression in six mouse groups fed individual dietary regimens. The histogram shows the number of enterocytes (*y* axis), as a percentage of the total cell count, and the intensity of Aβ signal (*x* axis) in mice fed low-fat (LF), high-cholesterol (HC), HC + ezetimibe, LF + ezetimibe, LF + atorvastatin and HC + atorvastatin dietary regimens. Values are means, with their standard errors represented by vertical bars. *P* value was determined with the χ^2 test and $P<0{\cdot}05$ considered significant. Staining intensity was considered negative when there was no immunostaining (0; □) and positive when mild (1+; □), moderate (2+; ■) and intense (3+; ■) Aβ staining was generated. Mice fed cholesterol had attenuated Aβ expression when compared with the LF group ($P<0{\cdot}0001$; Pearson's χ^2 85·206; df 3). Aβ expression was restored in cholesterol-supplemented mice given ezetimibe (HC + ezetimibe *v.* HC). Moreover, the atorvastatin-mediated increase in Aβ immunostaining in LF mice, compared with the LF diet group, was abolished by exogenous cholesterol feeding.

enterocytic Aβ[14]. To explore if this observation was specifically in response to dietary cholesterol, in the present study we determined enterocytic Aβ expression in 6-week-old female wild-type mice given a LF diet free of saturated fat but supplemented with cholesterol. The effect of cholesterol on enterocytic Aβ homeostasis was also investigated by pharmacologically blocking dietary cholesterol absorption and endogenous cholesterol biosynthesis.

For all groups of mice, the majority of Aβ immunostaining was found concentrated within the perinuclear region of the enterocytes as previously reported[14]. The distribution was reminiscent of cell-culture studies, which showed substantial Aβ within the endoplasmic reticulum and the Golgi apparatus[7–9]. The enterocytic perinuclear Aβ distribution is

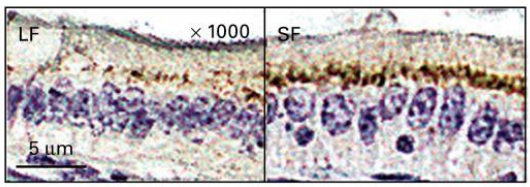


Fig. 3. Amyloid-β (Aβ) expression in enterocytes of wild-type mice fed low-fat (LF) and 20 % saturated fat (SF) diets. The enterocytes of mice given a LF diet had modest Aβ abundance within the perinuclear region of the cell compared with mice given a SF-enriched diet. Staining for Aβ is shown at high magnification (× 1000; scale bar 5 μm) in brown and the cell nuclei in blue.

consistent with the sites of chylomicron production and we previously hypothesised that high-fat feeding stimulates Aβ secretion in association with nascent lymph chylomicrons[14].

In contrast to our hypothesis, in the present study we demonstrate that enterocytic Aβ concentration was attenuated in response to cholesterol feeding. Our findings are consistent with that of Howland *et al.* [35], who established that exposure to increased dietary cholesterol resulted in a significant reduction in the brain level of $A\beta_{1-40/42}$ in AβPP gene-targeted mice. In the present study, reduced enterocytic Aβ abundance as a consequence of cholesterol feeding may be a reflection of attenuated Aβ biosynthesis and/or enhanced secretion, probably associated with intestinally derived lipoproteins[30]. Consistent with the latter, exogenous cholesterol has been found to stimulate chylomicron biogenesis and secretion[28]. Our previous studies showed increased cellular Aβ[14] in response to high saturated fat and cholesterol feeding. In the present study, we confirm that saturated fat feeding induces enterocytic Aβ accumulation.

The attenuation of enterocytic Aβ accumulation in cholesterol-supplemented mice could be abolished by the administration of ezetimibe, which effectively suppresses cholesterol absorption[36]. Changes in plasma cholesterol concentration were not observed with cholesterol feeding and ezetimibe treatment; however, enterocytic abundance of cholesterol may nonetheless have occurred. There was no difference in enterocytic Aβ staining intensity in mice given cholesterol-free chow plus ezetimibe, suggesting that no pleiotropic effects occurred with this agent.

The suppressive effect of dietary cholesterol on enterocytic Aβ abundance occurred in the absence of significant changes of plasma cholesterol, indicating that the dose of sterol provided was within physiologically tolerable limits. This contrasts with our previous studies with mice given saturated fats plus cholesterol[14] and studies by others[15,35] where higher doses of sterol supplementation significantly increased plasma cholesterol concentration. The absence of significant changes in plasma cholesterol homeostasis with an attenuation of enterocytic Aβ is consistent with the concept of reduced Aβ production, rather than enhanced secretion associated with chylomicrons. Collectively, these data support the notion that exogenous cholesterol plays an important role in Aβ homeostasis in the absorptive epithelial cells of the small intestine.

To explore whether endogenous cholesterol biosynthesis regulates enterocytic Aβ homeostasis, mice were given atorvastatin, a potent cholesterol biosynthesis inhibitor. Consistent with the findings of exogenous cholesterol attenuating Aβ concentration in enterocytes, we found that inhibiting cholesterol synthesis with atorvastatin significantly enhanced Aβ. Our findings are an extension on the findings by Park *et al.* [37] who demonstrated cholesterol biosynthesis inhibition by lovastatin increased Aβ generation in the brain tissues of female transgenic mice with familial Alzheimer's disease.

In the present study, we cannot ascertain the mechanism for the atorvastatin-induced effect. Plasma cholesterol, whilst not changed in LF mice given atorvastatin, is a poor surrogate marker of epithelial cell cholesterol homeostasis, because plasma cholesterol is mainly of hepatic origin. However, clues as to whether the atorvastatin effect was pleiotropic are indicated when the drug was co-administered with

exogenous cholesterol (HC + atorvastatin). We observed that dietary cholesterol abolished the atorvastatin effect on enterocytic Aβ homeostasis, consistent with this agent regulating enterocytic Aβ concentration *via* modulation of enterocytic pools of cholesterol.

Mechanisms by which dietary cholesterol inhibits enterocytic Aβ concentration are not readily explained. Frears *et al.* [38] observed that in the presence of cholesterol, human AβPP-transfected human embryonic kidney (HEK) cells secrete greater quantities of Aβ. In contrast, Abad-Rodriguez *et al.* [39] showed that upon lowering cholesterol, Aβ generation was increased in primary cell cultures of rat embryo hippocampal neurones and also identified that a moderate reduction in membrane cholesterol resulted in increased β-secretase. When cholesterol was added back to the cell-culture medium, β-secretase level returned to a similar level as the control. In animal model studies, enhanced intracellular Aβ accumulation was evident in brain tissues of cholesterol-fed rabbits[18], but this probably reflected deposition rather than intracellular abundance. Increased cerebral Aβ deposition was also reported in TgAPPsw mice with dietary induced hypercholesterolaemia[17], but intestinal Aβ expression in this animal model system has not been reported. Our findings suggest that reduced cholesterol availability enhances enterocytic Aβ abundance by enhancing its production.

Increased cellular cholesterol could act to increase membrane rigidity of intracellular compartments and thereby block accessibility of secretases to AβPP[39]. Furthermore, intracellular distribution between non-esterified cholesterol in the membrane and cholesteryl esters in the cytoplasm may be important in modulating Aβ homeostasis by alternative mechanisms. Inhibition of cholesterol trafficking in neuronal cells decreased β-secretase but enhanced γ-secretase processing of AβPP[40]. The substantial increase in γ-secretase resulted in an increased concentration of intracellular Aβ[40]. It is possible that an alteration in subcellular cholesterol distribution might induce changes in the cell membranes of intracellular compartments, endoplasmic reticulum and Golgi apparatus, and re-localise the enzymes responsible for Aβ production. Cholesteryl esters also appear pivotal to chylomicron biogenesis[24,25] and may influence secretion of Aβ by modulating release of the native particles. Moreover, there is some evidence of reciprocal modulation of acyl-coenzyme A:cholesterol acyltransferase and Aβ biosynthesis in cultured cell models[41].

The findings of the present study could suggest that cholesterol absorption inhibitors and 3-hydroxy-3-methylglutaryl CoA reductase inhibitors would lead to higher intestinal Aβ concentrations and therefore to a higher risk of Alzheimer's disease. However, the present study is not designed to consider the potential benefits or risks associated with the use of cholesterol-modulating agents on Alzheimer's disease risk. Inhibitors of 3-hydroxy-3-methylglutaryl CoA reductase reduce CVD and exhibit pleiotropic effects independent of lipid modification and, similarly, some lipid-modulating agents may improve outcome or ameliorate symptoms of neurological disorders[23,42]. Nonetheless, the appropriateness of initiating statin therapy is not presently established[43]. Elevated total cholesterol, LDL and TAG with normal HDL and total-cholesterol:HDL ratio characterise the lipid profile in Alzheimer's disease. However, scores on the mini mental-state examination do not correlate with lipid parameters, suggesting no interaction between cholesterol and cognition in Alzheimer's disease[44]. On the other hand, population studies support a role of dietary fats in Alzheimer's disease. Laitinen *et al.* [45] reported that intake of unsaturated fats is protective, whereas intake of saturates increases the risk of Alzheimer's disease. In the Framingham study, the top quartile of plasma DHA was associated with a 47 % reduction in risk of all-cause dementia[46]. Furthermore, evidence continues to come from animal studies including that of Oksman *et al.* [47], which confirmed that saturates increased, while DHA decreased, Aβ levels compared with a soya oil diet. Also, in cell-culture studies, fatty acids increased presenilin 1, γ-secretase and Aβ independent of any increase in cellular cholesterol[48]. Investigating the putative effects of dietary fatty acids on intestinal Aβ homeostasis may be worthwhile exploring.

Collectively, the findings of the present study indicate that enterocytic Aβ concentration is differentially regulated by dietary cholesterol and saturated fats. Dietary induced changes in production and secretion of Aβ may influence the net circulating pool of Aβ and, possibly, bi-directional kinetics of Aβ across the blood–brain barrier.

Acknowledgements

The authors declare no conflict of interest. The present study was financially supported by the Australian Technology Network Centre for Metabolic Fitness (Curtin University node).

M. M. P.-G. contributed to experimental data collection and to the writing of the manuscript. S. G. contributed to experimental data collection and to the writing of the manuscript. R. J. contributed to the development of methods and to the writing of the manuscript. L. J. contributed to the project concept, experimental design and to the writing of the manuscript. S. D. contributed to the experimental design and statistical assessment. J. C. L. M. contributed to the project concept, research funding, experimental design, data collection and to the writing of the manuscript.

References

1. Selkoe DJ (1994) Cell biology of the amyloid β-protein precursor and the mechanism of Alzheimer's disease. *Annu Rev Cell Biol* **10**, 373–403.
2. Fukuoka A, Nakayama H & Doi K (2004) Immunohistochemical detection of β-amyloid and β-amyloid precursor protein in the canine brain and non-neuronal epithelial tissues. *J Protein Folding Disord* **1**, 173–178.
3. Golde TE & Eckman CB (2001) Cholesterol modulation as an emerging strategy for the treatment of Alzheimer's disease. *Drug Discov Today* **6**, 1049–1055.
4. Haass C, Hung AY, Schlossmacher MG, Oltersdorf T, Teplow DB & Selkoe DJ (1993) β-Amyloid peptide and a 3-kDa fragment are derived by distinct cellular mechanisms. *J Biol Chem* **268**, 3021–3024.
5. Golde TE, Estus S, Younkin LH, Selkoe DJ & Younkin SG (1992) Processing of the amyloid protein-precursor to potentially amyloidogenic derivatives. *Science* **255**, 728–730.
6. Haass C, Koo EH, Mellon A, Hung AY & Selkoe DJ (1992) Targeting of cell-surface β-amyloid precursor protein to lysosomes: alternative processing into amyloid-bearing fragments. *Nature* **357**, 500–503.

7. Greenfield JP, Tsai J, Gouras GK, Hai B, Thinakaran G, Checler F, Sisodia SS, Greengard P & Xu H (1999) Endoplasmic reticulum and trans-Golgi network generate distinct populations of Alzheimer β-amyloid peptides. *Neurobiol* **96**, 742–747.
8. Turner RS, Suzuki N, Chyung ASC, Younkin SG & Lee WMY (1996) Amyloids β40 and β42 are generated intracellularly in cultured human neurons and their secretion increases with maturation. *J Biol Chem* **271**, 8966–8970.
9. Wild-Bode C, Yamazaki T, Capell A, Leimer U, Steiner H, Ihara Y & Haass C (1997) Intracellular generation and accumulation of amyloid β-peptide terminating at amino acid 42. *J Biol Chem* **272**, 16085–16088.
10. Deane R, Sagare A, Hamm K, Parisi M, LaRue B, Guo H, Wu Z, Holtzman DM & Zlokovic BV (2005) IgG-assisted age-dependent clearance of Alzheimer's amyloid β-peptide by the blood–brain barrier neonatal Fc receptor. *J Neurosci* **25**, 11495–11503.
11. Mackic JB, Bading J, Ghiso J, Walker L, Wisniewski T, Frangione B & Zlokovic BV (2002) Circulating amyloid β-peptide crosses the blood–brain barrier in aged monkeys and contributes to Alzheimer's disease lesions. *Vascul Pharmacol* **38**, 308–313.
12. Goldgaber D, Harris HW, Hla T, Maciag T, Donnelly RJ, Jacobsen JS, Vitek MP & Gajdusek DC (1989) Interleukin 1 regulates synthesis of amyloid β-protein precursor mRNA in human endothelial cells. *Proc Natl Acad Sci U S A* **86**, 7696–7710.
13. Chen M, Inestrosa NC, Ross GS & Fernandez HL (1995) Platelets are the primary source of amyloid β-peptide in human blood. *Biochem Biophys Res Commun* **213**, 96–103.
14. Galloway S, Jian L, Johnsen R, Chew S & Mamo JCL (2007) β-Amyloid or its precursor protein is found in epithelial cells of the small intestine and is stimulated by high-fat feeding. *J Nutr Biochem* **18**, 279–284.
15. George AJ, Holsinger RMD, McLean CA, Laughton KM, Beyreuther K, Evin G, Matsters CL & Li Q (2004) APP intracellular domain is increased and soluble Aβ is reduced with diet-induced hypercholesterolemia in a transgenic mouse model of Alzheimer disease. *Neurobiol Dis* **16**, 124–132.
16. Refolo LM, Malester B, LaFrancois J, Bryant-Thomas T, Wang R, Tint GS, Sambamurti K, Duff K & Papolla MA (2000) Hypercholesterolemia accelerates the Alzheimer's amyloid pathology in a transgenic mouse model. *Neurobiol Dis* **7**, 321–331.
17. Shie F, Jin L, Cook DG, Leverenz JB & LeBoeuf RC (2002) Diet-induced hypercholesterolemia enhances brain Aβ accumulation in transgenic mice. *Neuroreport* **13**, 455–459.
18. Sparks DL, Scheff SW, Hunsaker JC III, Liu H, Landers T & Gross DR (1994) Induction of Alzheimer-like β-amyloid immunoreactivity in the brains of rabbits with dietary cholesterol. *Exp Neurol* **126**, 88–94.
19. Giri R, Selvaraj S, Miller CA, Hofman F, Yan SD, Stern D, Zlokovic BV & Kalra VK (2002) Effect of endothelial cell polarity on β-amyloid-induced migration of monocytes across normal and AD endothelium. *Am J Physiol Cell Physiol* **283**, C895–C904.
20. LaRue R, Hogg E, Sagare A, Jovanovic S, Maness L, Maurer C, Deane R & Zlokovic BV (2004) Method for measurement of the blood–brain barrier permeability in the perfused mouse brain. *J Neurosci Methods* **138**, 233–242.
21. Sparks DL (1996) Intraneuronal β-amyloid immunoreactivity in the CNS. *Neurobiol Aging* **17**, 291–299.
22. Fassbender K, Simons M, Bergmann C, *et al.* (2001) Simvastatin strongly reduces levels of Alzheimer's disease β-amyloid peptides $A\beta_{42}$ and $A\beta_{40}$ *in vitro* and *in vivo*. *Proc Natl Acad Sci U S A* **98**, 5856–5861.
23. Wolozin B (2004) Cholesterol, statins and dementia. *Curr Opin Lipidol* **15**, 667–672.
24. Shen H, Howles P & Tso P (2001) From interaction of lipidic vehicles with intestinal epithelial cell membranes to the formation and secretion of chylomicrons. *Advanced Drug Del Rev* **50**, S102–S125.
25. Simons K & Ikonen E (2000) How cells handle cholesterol. *Science* **290**, 1721–1726.
26. Mahmood-Hussain M (2000) A proposed model for the assembly of chylomicrons. *Atherosclerosis* **148**, 1–15.
27. Cesar TB, Oliveira MRM, Mesquita CH & Maranhao RC (2006) High cholesterol intake modifies chylomicron metabolism in normolipidemic young men. *J Nutr* **136**, 971–976.
28. Pal S, Allister E, Thomson A & Mamo JCL (2002) Cholesterol esters regulate $apoB_{48}$ secretion in $CaCo_2$ cells. *Atherosclerosis* **161**, 55–63.
29. James AP & Mamo JCL (2005) The immunodetection of lipoprotein-bound amyloid-β is attenuated because of the presence of lipids. *Ann Clin Biochem* **42**, 70–72.
30. James AP, Pal S, Gennat HC, Vine DF & Mamo JCL (2003) The incorporation and metabolism of amyloid-β into chylomicron-like lipid emulsions. *J Alz Dis* **5**, 179–188.
31. Sudhop T, Lütjohann D, Kodal A, Igel M, Tribble DL, Shah S, Perevozskaya I & von Bergmann K (2002) Inhibition of intestinal cholesterol absorption by ezetimibe in humans. *Circulation* **106**, 1943–1948.
32. Davis HR Jr, Zhu L, Hoos LM, *et al.* (2004) Niemann-Pick C1 Like 1 (NPC1L1) is the intestinal phytosterol and cholesterol transporter and a key modulator of whole-body cholesterol homeostasis. *J Biol Chem* **279**, 33586–33592.
33. Naoumova RP, Dum S, Rallidis L, Abu-Muhana O, Neuwirth C, Rendell NB, Taylor GW & Thompson GR (1997) Prolonged inhibition of cholesterol synthesis explains the efficacy of atorvastatin. *J Lipid Res* **38**, 1496–1500.
34. Mikaelian I, Nanney LB, Parman KS, *et al.* (2004) Antibodies that label paraffin-embedded mouse tissues: a collaborative endeavor. *Toxic Path* **32**, 181–191.
35. Howland DS, Trusko SP, Savage MJ, *et al.* (1998) Modulation of secreted β-amyloid precursor protein and amyloid β-peptide in brain by cholesterol. *J Biol Chem* **273**, 16576–16582.
36. Altmann SW, Davis HR, Zhu L, *et al.* (2004) Niemann-Pick C1 like 1 protein is critical for intestinal cholesterol absorption. *Science* **303**, 1201–1204.
37. Park I, Hwang EM, Honga HS, Boo JH, Oh SS, Lee J, Jung MW, Bang OY, Kim SU & Mook-Jung I (2003) Lovastatin enhances Aβ production and senile plaque deposition in female Tg2576 mice. *Neurobiol Aging* **24**, 637–643.
38. Frears ER, Stephens DJ, Walters CE, Davies H & Austen BM (1999) The role of cholesterol in the biosynthesis of β-amyloid. *Neuroreport* **10**, 1699–1705.
39. Abad-Rodriguez J, Ledesma MD, Craessaerts K, Perga S, Medina M, Delacourte A, Dingwall C, Strooper BD & Dotti CG (2004) Neuronal membrane cholesterol loss enhances amyloid peptide generation. *J Clin Biochem* **167**, 953–960.
40. Runz H, Rietdorf J, Tomic I, de Bernard M, Beyreuther K, Pepperkok R & Hartmann T (2002) Inhibition of intracellular cholesterol transport alters presenilin localisation and amyloid precursor protein processing in neuronal cells. *J Neurosci* **22**, 1679–1689.
41. Puglielli L, Konopka G, Pack-Chung E, Ingano LAM, Berezovska O, Hyman BT, Chang TY, Tanzi RE & Kovacs DM (2001) Acyl-coenzyme A: cholesterol acyltransferase modulates the generation of the amyloid β-peptide. *Nature Cell Biol* **3**, 905–912.
42. Petanceska SS, DeRosa S, Olm V, Diaz N, Sharma A, Thomas-Bryant T, Duff K, Pappolla M & Refolo LM (2002) Statin therapy for Alzheimer's disease: will it work? *J Mol Neurosci* **19**, 155–161.
43. Reiss AB & Wirkowski E (2007) Role of HMG CoA reductase inhibitors in neurological disorders: progress to date. *Drugs* **67**, 2111–2120.

NS British Journal of Nutrition

44. Sabbagh M, Zahiri HR, Ceimo J, Cooper K, Gaul W, Connor D & Sparks DL (2004) Is there a characteristic lipid profile in Alzheimer's disease? *J Alzheimers Dis* **6**, 585–589.
45. Laitinen MH, Ngandu T, Rovio S, Helkala EL, Uusitalo U, Viitanen M, Nissinen A, Tuomilehto J, Soininen H & Kivipelto M (2006) Fat intake at midlife and risk of dementia and Alzheimer's disease: a population-based study. *Dement Geriatr Cogn Disord* **22**, 99–107.
46. Schaefer EJ, Bongard V, Beiser AS, Lamon-Fava S, Robins SJ, Au R, Tucker KL, Kyle DJ, Wilson PW & Wolf PA (2006) Plasma phosphatidylcholine docosahexanoic acid content and risk of dementia and Alzhimer disease: the Framingham Heart Study. *Arch Neurol* **63**, 1545–1550.
47. Oksman M, Iivonen H, Hogyes E, Amtul Z, Penke B, Leenders I, Broersen L, Lutjohann D, Hartmann T & Tanila H (2006) Impact of different saturated fatty acid, polyunsaturated fatty acid and cholesterol containing diets on β-amyloid accumulation in APP/PS1 transgenic mice. *Neurobiol Dis* **23**, 563–572.
48. Liu Y, Yang L, Conde-Knape K, Beher D, Shearman MS & Shacter NS (2004) Fatty acids increase presenilin-1 levels and γ-secretase activity in PSwt-1 cells. *J Lipid Res* **45**, 2368–2376.

NS British Journal of Nutrition

Article 3:

Pallebage-Gamarallage, M. M., **S. Galloway**, R. Takechi, S. Dhaliwal, and J. C. Mamo. "Probucol Suppresses Enterocytic Accumulation of Amyloid-Beta Induced by Saturated Fat and Cholesterol Feeding." [In eng]. *Lipids* 47, no. 1 (Jan 2012): 27-34.

Synopsis:

The manuscript titled "Probucol Suppresses Enterocytic Accumulation of Amyloid-Beta Induced by Saturated Fat and Cholesterol Feeding." explores the putative effects of saturated fat and probucol on small intestine derived Aβ. The article directly reinforced my findings and also puts forward a possible therapeutic drug, probucol, that when incorporated into a SFA diet, significantly attenuates enterocytic Aβ. Dietary fat and drugs which facilitate apo B-lipoprotein production, secretion and plasma kinetics can also modulate enterocytic Aβ production and secretion and therefore contribute toward AD risk. I contributed to the article by assisting in the appraisal of the manuscript, animal care and collection of samples.

Background:

Co-localisation of apo B with amyloid plaques in post mortem brain samples from AD humans (Namba et al. 1992) and transgenic mouse models (Takechi et al. 2010b) presents direct evidence that peripheral Aβ-apo B contributes to cerebral amyloid deposits in the presence of BBB damage. In AD subjects (Mamo et al. 2008), the plasma concentration of Aβ-lipoprotein is increased and transgenic animal research suggests that increased secretion of Aβ-lipoprotein may occur early during the pathological process in AD (Burgess et al. 2006). Probucol is a cholesterol lowering agent and has lipid-lowering properties which have been attributed to its influence on secretion and clearance of apo B-lipoproteins (Mamo et al. 1993, Gershkovich and Hoffman 2005). Probucol becomes incorporated into nascent lipoprotein molecules and has been effective in lowering the rate of cognitive decline in clinical studies (Poirier 2003, Poirier 2005) as well as reducing the rate of cerebral amyloid deposits in transgenic mice (Poirier 2003); although mechanisms of action were not clear. It is possible that the beneficial effects of

probucol were extended or were directly influenced by modulation of abundance and plasma kinetics of apo B-lipoprotein-Aβ from enterocytes.

Methods:

Intestinal Aβ-lipoprotein homeostasis was explored using wild-type C57BL/6J mice fed HF diet (15 % w/w as saturated fat with 1 % cholesterol) and LF diet (<4 % fat as unsaturated fat and no cholesterol) for 1 month. Probucol was incorporated into both LF and HF diets at 1 % (w/w). Double immunofluorescence using two polyclonal antibodies was performed as previously described (Takechi et al. 2008). Three-dimensional quantitative analysis and co-localization of enterocytic Aβ and apo B signal was performed by Automatic Measurement Program (AxiVision v 4.7.1).

Results:

Apo B and Aβ were found within the perinuclear location of enterocytes as well as within the lacteals of the mucosa layer of the small intestine. This staining pattern was observed for all groups and the net abundance (perinuclear + lacteal) was not significantly different between groups; however, enterocytic abundance and secretion modulation effects were present. Compared with the HF group, the HF + probucol treatment group showed attenuated enterocytic Aβ which paralleled an increase in lacteal staining of apo B. The location of intestinal Aβ and apo B appeared to be qualitatively comparable indicating that Aβ-apo B binding and secretion occurs in complex. Although this is the case, there was about 70-80 % more Aβ in the perinuclear location of enterocytes in contrast to approximately 80 % of apo B within the lacteals. Compared to LF feeding, HF feeding induced a 2-fold increase in Aβ which was predominantly observed within the perinuclear location whilst secretion of Aβ remained relatively unchanged. Probucol did not influence Aβ abundance under LF feeding but caused a significant decrease in cellular Aβ under HF feeding with no change observed in lacteal Aβ abundance. In both LF and HF groups, probucol decreased enterocytic Aβ and increased lacteal Aβ indicating stimulatory effects on secretion of apo B; HF feeding had greater stimulatory effect as probucol. In addition, 1% probucol supplementation was sufficient to lower plasma cholesterol and increase body weight in both LF and HF groups.

Discussion:

High fat feeding consistently increases enterocytic abundance of Aβ, which is greater than the suppressive effects of cholesterol. SFA can influence the metabolism of Aβ via increased Aβ production and association with apo B containing lipoproteins and/or its cellular degradation. This study suggests that probucol can lower enterocytic Aβ; a mechanism which could translate to reduced plasma Aβ levels and reduced risk of cerebral accumulations. Probucol may also benefit AD sufferers by protecting the BBB from oxidative damage and by reducing plasma lipids and pro-inflammatory proteins such as Aβ.

Lipids (2012) 47:27–34
DOI 10.1007/s11745-011-3595-4

ORIGINAL ARTICLE

Probucol Suppresses Enterocytic Accumulation of Amyloid-β Induced by Saturated Fat and Cholesterol Feeding

Menuka M. Pallebage-Gamarallage · Susan Galloway · Ryusuke Takechi · Satvinder Dhaliwal · John C. L. Mamo

Received: 7 February 2011 / Accepted: 7 July 2011 / Published online: 31 July 2011

Abstract Amyloid-β (Aβ) is secreted from lipogenic organs such as intestine and liver as an apolipoprotein of nascent triacylglycerol rich lipoproteins. Chronically elevated plasma Aβ may compromise cerebrovascular integrity and exacerbate amyloidosis—a hallmark feature of Alzheimer's disease (AD). Probucol is a hypocholesterolemic agent that reduces amyloid burden in transgenic amyloid mice, but the mechanisms for this effect are presently unclear. In this study, the effect of Probucol on intestinal lipoprotein-Aβ homeostasis was explored. Wild-type mice were fed a control low-fat diet and enterocytic Aβ was stimulated by high-fat (HF) diet enriched in 10% (w/w) saturated fat and 1% (w/w) cholesterol for the duration of 1 month. Mice treated with Probucol had the drug incorporated into the chow at 1% (w/w). Quantitative immunofluorescence was utilised to determine intestinal apolipoprotein B (apo B) and Aβ abundance. We found apo B in both the perinuclear region of the enterocytes and the lacteals in all groups. However, HF feeding and Probucol treatment increased secretion of apo B into the lacteals without any change in net villi abundance. On the other hand, HF-induced enterocytic perinuclear Aβ was significantly attenuated by Probucol. No significant changes in Aβ were observed within the lacteals. The findings of this study support the notion that Probucol suppresses dietary fat induced stimulation of Aβ biosynthesis and attenuate availability of apo B lipoprotein-Aβ for secretion.

Keywords Apolipoprotein B · Amyloid-β · Probucol · Small intestine · Saturated fat · Cholesterol

Abbreviations

AD	Alzheimer's disease
Aβ	Amyloid-β
Apo B	Apolipoprotein B
HF	High-fat
LF	Low-fat
TAG	Triacylglycerol(s)

Introduction

Alzheimer's disease (AD) is the most common cause of dementia, characterized by neuronal cell loss and amyloid-beta (Aβ) deposition on extracellular matrices and within the cerebrovasculature [1]. Amyloid-β is present at sub-nanomolar levels in most biological fluids, such as cerebrospinal fluid and plasma [2] and, at physiological levels, regulates cell growth [3–5]. A hydrophobic protein, Aβ may undergo oligomerisation when it becomes disassociated from chaperone proteins that ordinarily facilitate kinetics and metabolism. It is the fibrillar form of Aβ that is thought to trigger pro-inflammatory pathways that compromise neuronal integrity [6, 7].

The origin of cerebrovascular Aβ deposits in AD is presently unclear. Amyloid-β may be generated as a

M. M. Pallebage-Gamarallage · S. Galloway · R. Takechi · S. Dhaliwal · J. C. L. Mamo
School of Public Health, Curtin University of Technology, GPO Box U1987, Perth, WA 6845, Australia

M. M. Pallebage-Gamarallage · S. Galloway · R. Takechi · J. C. L. Mamo
Australian Technology Network, Centre for Metabolic Fitness, Perth, Australia

J. C. L. Mamo (✉)
Department of Health Sciences, Curtin University of Technology, Kent Street, Bentley, WA 6102, Australia
e-mail: J.Mamo@Curtin.edu.au

consequence of proteolytic processing of the amyloid-precursor-protein, which is expressed in significant quantities on the plasma membrane of neuronal cells [8]. However, biogenesis of Aβ is not increased in sporadic and late onset AD—the most common phenotype of AD [9, 10]. Rather, accumulating evidence suggests that enhanced blood-to-brain delivery relative to efflux, or via Aβ degradative pathways within the choroid plexus results in extracellular retention of Aβ and thereafter, inflammatory sequelae [11, 12].

Significant Aβ in blood is associated with apolipoprotein B (apo B) lipoproteins, particularly those enriched in triacylglycerol (TAG). Subjects with AD have greater apo B lipoprotein-Aβ relative to age-matched controls [13], and in transgenic amyloid mice, onset and progression of cerebral amyloidosis is associated strongly with the secretion into and concentration of plasma apo B lipoprotein-Aβ [14]. Apolipoprotein B immunoreactivity is evident in parenchymal amyloid plaque from human cadaver specimens [15]; and in Aβ-transgenic mice, cerebral apo B distribution and abundance strongly colocalise with extracellular deposits of Aβ [16]—observations consistent with a vascular contribution to disease aetiology.

A range of lipoprotein (lipid)-lowering agents are commonly used in clinical practice for the prevention and treatment of cardiovascular disease and may reduce AD risk by reducing cerebrovascular exposure to apo B lipoprotein-Aβ. The hydroxy-methyl-glutaryl coenzyme A reductase inhibitors lower plasma cholesterol by enhancing apo B lipoprotein clearance via high affinity receptor pathways and inhibiting apo B lipoprotein biogenesis [17–19]. Similarly, fibrates reduce plasma TAG by suppressing lipogenesis, a driver for the secretion of apo B lipoproteins [20]. Population and clinical studies generally support a risk reduction for all forms of dementia in subjects taking lipid-lowering agents [21, 22], although the mechanisms for this association are not clear.

Probucol is an older generation cholesterol-lowering agent that reduces plasma cholesterol by enhancing uptake via receptor pathways [23, 24]. However, other properties of Probucol make this a particularly interesting lipid-lowering agent relative to the plasma kinetics and metabolism of apo B-lipoprotein-Aβ. Probucol is hydrophobic and secreted into blood incorporated within the nascent TAG-rich apo B lipoproteins, a phenomenon that may influence Aβ association with, and secretion of, these macromolecules [23, 25]. In addition, lipoproteins that contain Probucol are cleared from circulation almost exclusively by the liver and consequently vascular retention is substantially reduced [23]. A small clinical study suggested that Probucol reduced cognitive decline in subjects with mild cognitive impairment [26, 27] and, consistent with the human findings, studies in transgenic amyloid mice showed that Probucol reduced the severity of amyloidosis [26]. In the latter, enhanced Aβ-efflux was put forward as one possible mechanism for the Probucol-induced effects.

The putative effects of Probucol on Aβ biogenesis and lipoprotein synthesis in lipogenic organs have not been reported. This study utilised an in vivo high-fat (HF) feeding model previously shown to stimulate enterocytic abundance of Aβ, to determine if Probucol modulates the secretion of apo B lipoprotein-Aβ from absorptive epithelial cells of the small intestine—a major site of Aβ biosynthesis [28–30].

Methods and Materials

Animals and Diet Conditions

The Curtin University Animal Experimentation and Ethics Committee approved the animal housing, handling and experimental procedures described. Seven-week-old female wild-type mice (C57BL/6J) were housed in groups and randomised into the diet or drug treatment groups ($n = 8$ mice per group). All mice were maintained on a 12-h light and dark cycle room, at 22°C and with free access to water and food. The low-fat control diet was standard AIN93M rodent chow containing <4% (w/w) fat as polyunsaturates, with <1% total digestible energy as lipids and was free of cholesterol (Glen Forrest Stockfeeders, Perth, Western Australia). To stimulate enterocytic Aβ production, the control feed was replaced with a HF diet enriched in saturated fats 10% (w/w) and 1% (w/w) cholesterol (Glen Forrest Stockfeeders). The principal fatty acid types in the HF treatment group were palmitic (16:0) and stearic (18:0) (total of 13% w/w) and oleic acid (18:1n-9, 6% w/w). Mice treated with Probucol (Sanofi-Aventis, Paris, France) had the drug incorporated into the chow at 1% (w/w) at the time of feed manufacture in order to achieve an estimated dose rate of 30 mg/day [23].

Tissue Collection and Sample Preparation

Mice were maintained for 32 days on the diets indicated and weighed weekly. Thereafter, mice were anaesthetised with pentobarbitone (45 mg/kg i.p.) and exsanguinated by cardiac puncture. Blood was collected into heparin tubes and stored in ice. Plasma was separated by short-speed centrifugation at 4°C and stored at −80°C.

A 2 cm segment of the small intestine duodenum at the proximal end was isolated, flushed with chilled phosphate buffer saline (PBS, pH 7.4) and fixed in 10% buffered formal saline for a minimum of 24 h. The tissues were then processed and longitudinal segments embedded in paraffin

wax. Serial sections of 5 μm thick were cut and mounted on silanised slides for histology and immunofluorescence.

Amyloid-β and Apolipoprotein B Immunofluorescence

Intestinal Aβ and apo B were detected by an immunofluorescent amplification method as previously described [31]. Intestinal tissue sections (5 μm) were deparaffinised, rehydrated and antigen-retrieval was carried out in boiling deionised water for 15 min. Briefly, all sections were permeabilised in PBS and incubated in blocking serum (20% goat serum).

For Aβ staining, polyclonal rabbit anti-human A$\beta_{1\text{-}40/42}$ antiserum (AB5076, Chemicon Temecula, CA), diluted to 1:2,000 in PBS was incubated overnight at 4°C. The specificity of the antibodies was previously established [28]. Sections were washed in PBS and incubated with biotinylated goat anti-rabbit secondary antibody (1:2,000 dilution) (E0432, DAKO, Carpentaria, CA) at room temperature for 1 h, followed by incubation with Streptavidin-Alexa Fluor® 546 (1:300 dilution) (S11225, Invitrogen, Victoria, Australia) for another hour in the dark for amplification. The nuclei were counterstained with DAPI (1:1,000 dilution) (Invitrogen, Victoria, Australia) for 5 min. The sections were then mounted with antifade mounting medium.

Enterocytic apo B was determined essentially as described for Aβ detection. Polyclonal rabbit anti-mouse apo B (ab20737, Abcam, Cambridge, UK) as primary and the biotinylated goat anti-rabbit secondary antibody was used at 1:2,250 dilutions.

Imaging

Digital images for photo microscopy were acquired through AxioCam HRm camera (Zeiss, Jena, Germany) with an AxioVert 200 M inverted microscope by Zeiss at 200× magnification (Plan Neofluar ×20 objective, 1.3 numerical aperture). Excitation and emission were achieved by using filters 43 (Ex BP545/25, beam splitter FT570 and Em BP605/70) and filter 49 (Ex G365, beam splitter FT395 and Em BP445/50) to determine fluorescence of Alexa Fluor® 546 and DAPI, respectively. Individual channels are free from fluorescence from other emission sources and are therefore clear of overlap. Each image was captured under identical exposure times utilising AxioVision software (version 4.7.1) to avoid artificial modification in pixel intensity.

Quantitative Immunofluorescent Imaging and Analysis

Images were collected at 200× magnification and approximately 30–50 images were captured per group showing at least four villi in each image (1,388 × 1,040 pixels per image). Pixel intensity for each fluorescent dye was obtained by calculating the densitometric sum by Automatic Measurement Program in AxioVision (Software version 4.7.1). Densitometric sum was calculated for each image staining intensity of Aβ, apo B and DAPI (nuclei).

For each image, either apo B or Aβ pixel intensities were standardised with total DAPI pixel intensity to normalise for cell number in the image, and expressed as per DAPI. Staining intensity in the perinuclear region within the enterocytes was calculated and expressed as perinuclear intensity per total DAPI for the image (perinuclear apo B/total DAPI, perinuclear Aβ/total DAPI). Lacteal staining intensity was obtained by subtracting perinuclear staining intensity from the whole villi intensity (lacteal apo B/total DAPI, lacteal Aβ/total DAPI). The data were then collated and final results are expressed as mean intensity ± standard error of mean per area unit.

Plasma Cholesterol and Triacylglycerol Analysis

Plasma Cholesterol and TAG were determined in duplicate by enzymatic assays (Randox Laboratories, Crumlin, UK) according to the manufacturers' instructions.

Statistical Analysis

All data was analysed by either parametric or non-parametric one-way analysis of variance (ANOVA) to assess the main effects of dietary fat and Probucol treatment and their two-way interactions. Post-hoc comparison of means was done if the associated main effect or interaction was statistically significant within the ANOVA procedure. P values < 0.05 were considered to be statistically significant.

Results

The distribution and abundance of immunoreactive apo B, an exclusive marker for nascent chylomicrons, was determined by quantitative immunofluorescent microscopy as previously described. Perinuclear enterocytic and lacteal abundance were utilized as surrogate markers of production and secretion, respectively. In all groups, the majority of immunoreactive apo B ($\approx$80%) was located within the lacteals (Fig. 1, 2a), indicative of the efficient packaging and secretory pathway of dietary lipids with chylomicrons. Provision of an HF diet for 32 days resulted in a 60% increase of secreted apo B commensurate with decreased perinuclear apo B (Fig. 1, 2a), but there was no significant change in net villi apo B abundance (perinuclear + lacteal). Incorporation of Probucol in the LF diet, like the HF

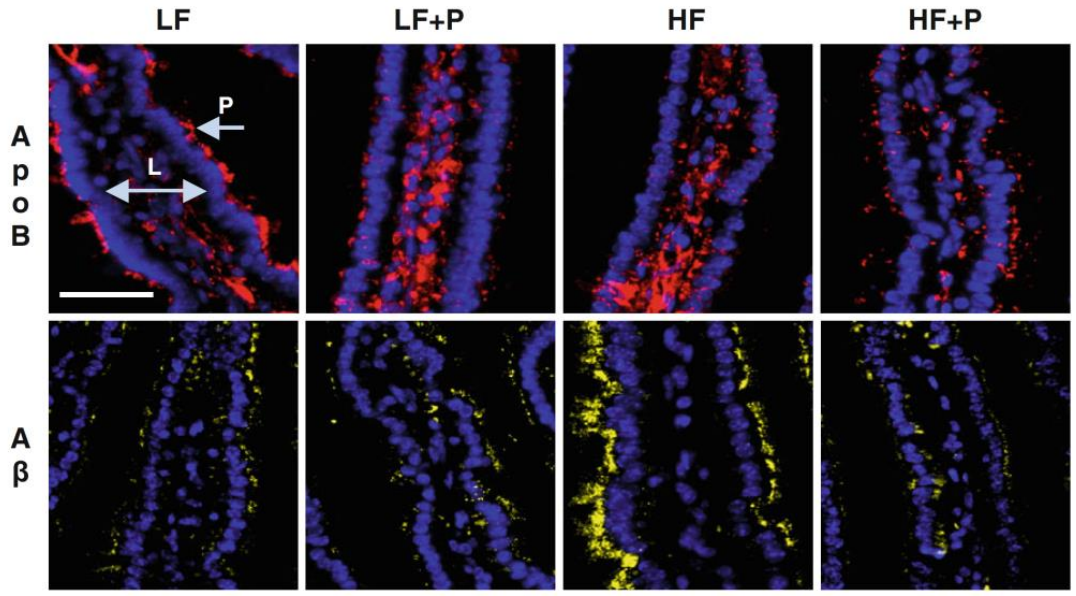


Fig. 1 Images showing apo B (*red*) and Aβ (*yellow*) in the intestinal villi. The nuclei are stained *blue*. The villi are lined with a single layer of absorptive epithelial cells. Amyloid-β staining is concentrated at the perinuclear region of the enterocytes (*P arrow*) with very little staining in the lacteals (*L arrow*). In contrast, a high concentration of the apo B is found within the lacteals of all the groups. *Bar* 30 μm, *LF* low-fat, *HF* high-fat, *P* probucol

diet, stimulated secretion of apo B. However, there did not appear to be a synergistic effect of HF + *P*. The findings of similar net villi abundance of apo B between treatment groups and a strong negative association between the perinuclear- versus lacteal-apo B distribution (Fig. 2c), is consistent with studies suggesting that enterocytic apo B is synthesized constitutively, whereas the secretion of the nascent lipoproteins is modifiable in response to the availability of dietary lipids [32].

The perinuclear and lacteal distribution of Aβ was qualitatively identical to that of apo B. Indeed, co-localisation analysis confirms that Aβ secreted from enterocytes was associated with chylomicrons. However, there were substantial differences in the relative villi abundance of Aβ and apo B and in the pattern of secretion between the two proteins. In contrast to apo B, approximately 70–80% of total intestinal villi Aβ was observed within the baso-lacteal nuclear region of the absorptive epithelial cells, suggesting that only small quantities of the total enterocytic Aβ pool were being secreted (Fig. 1, 2b). The HF-enriched diet doubled intestinal villi Aβ abundance but, in contrast to apo B, this was reflected predominantly in increased enterocytic abundance with virtually no change in the secreted component (Fig. 2b). Incorporation of Probucol in the HF diet normalized enterocytic Aβ to levels that were comparable to the LF control, in the absence of a reduction in lacteal Aβ. The latter suggests that Probucol reduced enterocytic Aβ primarily as a consequence of lower rates of biosynthesis. However, correlation analysis of perinuclear versus lacteal Aβ identified a relatively weak but nonetheless positive association (Fig. 2d), suggesting that increased rates of Aβ production also lead to modest increases in apo B lipoprotein-Aβ secretion.

The effects of HF- or Probucol-supplemented diets on plasma cholesterol, plasma TAG and body weight gain for each group of mice is given in Table 1. The mice fed the HF enriched diet had an increase in plasma cholesterol of more than two-fold compared to the LF control; however, the incorporation of Probucol completely abolished this effect. Indeed, the HF + *P* group had comparable plasma cholesterol to the LF + *P* treated mice. Probucol also significantly reduced plasma cholesterol in LF mice. In contrast, there was no appreciable effect of HF feeding, or Probucol, on plasma TAG in any treatment group.

Mice maintained on the HF diet were found to have a greater rate of body weight gain compared to mice maintained on the LF diet. Probucol had a synergistic stimulatory effect on body weight gain. Mice on LF + *P* were similar in weight to mice maintained on the HF diet, and mice on the HF + *P* were significantly heavier in weight than mice on HF alone.

Discussion

In absorptive epithelial cells of the small intestine, dietary fats regulate enterocytic abundance of Aβ profoundly, reflecting either changes in Aβ biogenesis, or in the

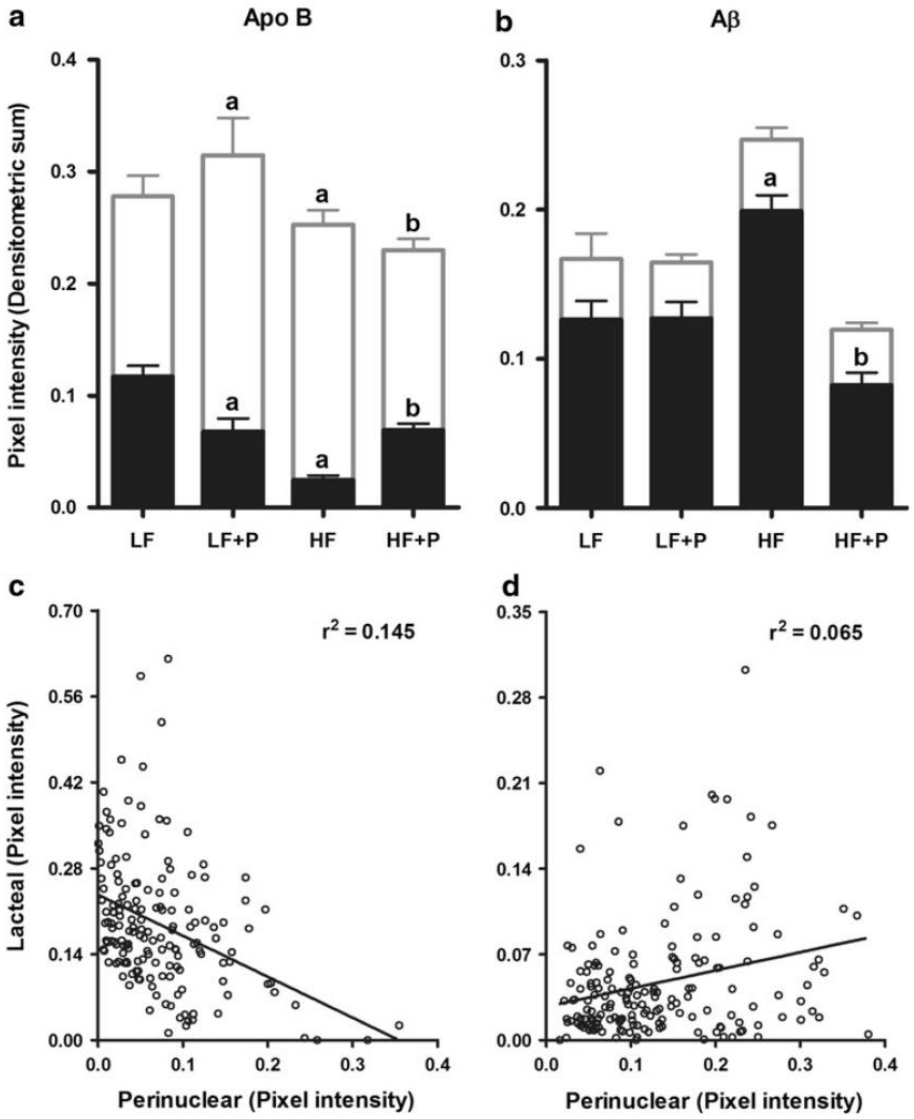


Fig. 2 **a**, **b** *Bar graphs* showing quantitative analysis of perinuclear (*filled squares*) and lacteal (*open squares*) apo B and Aβ staining intensities. Pixel intensity is expressed as densitometric sum as a ratio of nuclei intensity to normalise for nuclei number. **c**, **d** Scatter plots showing the distribution of apo B and Aβ between the perinuclear and lacteal regions of all groups. Pearson's correlation analysis (r^2 values) for apo B and Aβ was 0.145 ($P < 0.0001$) and 0.065 ($P = 0.0006$), respectively. **a** Statistically significant in comparison to the LF group ($P < 0.05$). **b** Statistically significant in comparison to the HF group ($P < 0.05$). *LF* low-fat, *HF* high-fat, *P* probucol

secretion of lipoproteins containing Aβ [28, 29]. Several lipid-lowering agents, including statins and fibrates, have been shown to suppress apo B secretion [17, 18, 20]; however, Probucol may have pleiotropic benefits post-secretion, including enhanced hepatic clearance apo B lipoprotein-Aβ and anti-oxidant activity.

In this study, an established in vivo murine model was used to investigate if Probucol modulates the effects of a HF diet on enterocytic Aβ and its secretion thereafter, with apo B lipoproteins. The study confirms that a HF diet substantially increases enterocytic perinuclear abundance of Aβ. Apolipoprotein B lipoprotein secretion is enhanced by HF feeding, but without evidence of a concomitant increase in lacteal Aβ staining. Therefore, the HF-mediated effect on enterocytic Aβ abundance is likely to be a consequence of greater rates of Aβ synthesis, rather than diminished rates of secretion. Previous studies reported that a HF-induced accumulation of enterocytic Aβ is progressively depleted in the post-absorptive state, or once food is withdrawn [28]. Hence, a dietary fat induced stimulation in Aβ biogenesis with constitutive rates of secretion as suggested in this study, would result in extended post-prandial amyloidemia. Clinical studies in normal healthy subjects consuming a mixed lipid meal are consistent with a transient single meal effect [34]. A phenomenon of extended exposure may be important in modulation vascular function. Co-administration of Probucol with the HF diet completely abolished the HF-induced effect on enterocytic Aβ abundance in the absence of a significant stimulatory effect on apo B lipoprotein-Aβ secretion. The findings are consistent with Probucol normalising enterocytic Aβ biogenesis, rather than promoting enterocytic secretion of Aβ.

Table 1 Effect of various feedings regimes on the average weight and plasma lipids in wild-type mice (C57BL/6J)

Feeding regime[c]	Body weight (g)[d]		Plasma lipids (mM)[e]	
	Final	Weight gain	TC	TAG
LF ($n = 8$)	20.56 ± 0.27	2.00 ± 0.16	1.86 ± 0.06	0.55 ± 0.05
LF + P ($n = 8$)	22.23 ± 0.36[a]	4.55 ± 0.16[a]	0.83 ± 0.04[a]	0.65 ± 0.06
HF ($n = 8$)	22.48 ± 0.33[a]	4.36 ± 0.19[a]	2.88 ± 0.17[a]	0.63 ± 0.10
HF + P ($n = 8$)	23.58 ± 0.32	5.82 ± 0.19[b]	0.67 ± 0.05[b]	0.39 ± 0.04

Data represented as mean ± standard error of the mean (SEM), numbers (n) indicate total number of samples used

LF low-fat, *HF* high-fat, *P* probucol, *TC* total serum cholesterol, *TAG* serum triglyceride

Values with lower case [a] and [b] indicate statistical significance in comparison with LF and HF groups, respectively, where $P < 0.05$

[c] Wild-type mice (C57BL/6J) were randomised to four different feeding regimens ($n = 8$ mice per group) and were fed their respective diets for 32 days. Weights, total serum cholesterol and triglyceride levels at the end of the experiment were compared between the groups with Post-hoc comparison of means within the ANOVA procedure and $P < 0.05$ was considered significant

[d] The average final body weights (per mouse) for LF + P and HF groups were significantly greater in comparison to the LF group; however, HF + P group final body weight was similar to that of the HF group. There was significant weight gain by all groups (vs LF) and there was also weight gain by the HF + P treated mice in comparison to the HF group

[e] Total plasma cholesterol level was significantly reduced by probucol treatment alone (vs LF), also by the HF + P treated group in comparison to the HF group. Fat feeding significantly have increased the circulating cholesterol level. On the other hand, neither the probucol treatment nor fat feeding did not affect the triglyceride concentration

Indeed, whilst Probucol was found to stimulate apo B lipoprotein secretion in LF-fed mice, there was no evidence that this translated into significantly increased enterocytic release of Aβ.

The HF diet utilized in this study contained both SFA and cholesterol, provided together in a context that it is physiologically relevant in comparison to commonly consumed atherogenic diets. The effects of Probucol on Aβ synthesis and secretion reported in this study must therefore be considered in the context of a mixed dietary lipid setting. Regulation by Probucol may change depending on the interactive effects of dose and duration of dietary lipids.

Several studies have shown synergistic stimulatory effects of fatty acids and cholesterol on apo B lipoprotein secretion [35, 36]. Therefore, the finding of increased apo B lipoprotein secretion shown in this study in HF-fed mice is to be expected. However, previous studies in the same strain of mice fed SFA, or cholesterol, found stimulatory and suppressive effects, respectively, on enterocytic abundance of Aβ [29, 33]. Saturated fatty acids were shown to have a profound stimulatory effect on enterocytic Aβ abundance, whereas dietary cholesterol was inhibitory. The reasons for the paradoxical differences between SFA and cholesterol have not been determined but may include differential regulation of Aβ biogenesis, transfer and association of Aβ with apo B lipoproteins and/or changes in intracellular degradation of Aβ or apo B lipoproteins.

In this study, saturated fats presented at 20% of digestible energy combined with 1% (w/w) cholesterol increased enterocytic abundance by approximately 75% above control mice given the LF diet alone. Hence, it would appear that the effects of SFA on enterocytic Aβ homeostasis were substantially greater than that of dietary cholesterol. How SFA influence Aβ biogenesis and association with apo B lipoproteins is not known. One possibility is increased lipidation of Aβ, a process found to protect other lipophilic apoproteins from proteolytic degradation.

Several studies suggest that the intracellular distribution between free cholesterol may be important in modulating Aβ homeostasis and intracellular kinetics. Inhibition of cholesterol trafficking in neuronal cells decreased β-secretase but enhanced γ-secretase processing of Aβ precursor protein [37]. The substantial increase in γ-secretase resulted in an increased intracellular concentration of Aβ [37]. Whilst in enterocytes Aβ biogenesis does not appear to occur at the plasma membrane, the subcellular distribution of cholesterol might nonetheless induce critical changes in the cell membranes of intracellular compartments such as within the endoplasmic reticulum and Golgi or re-localise enzymes responsible for Aβ synthesis, or its association with primordial lipoproteins. The notion that Probucol regulates enterocytic biogenesis of Aβ or association with apo B lipoproteins via modulation of intracellular pools of cholesterol is supported by the findings of Tawara et al. [38], who reported that Probucol stimulates cholesterol biosynthesis in absorptive epithelial cells of the small intestine, a process that would suppress Aβ biogenesis.

The HF diet resulted in greater body weight gain compared to LF-fed mice, presumably as a consequence of increased caloric intake and, somewhat surprisingly, Probucol also enhanced body weight gain in LF-fed mice. However, there was no evidence that body weight was associated with perinuclear or lacteal abundance of Aβ, or

of apo B lipoprotein-Aβ, so it is unlikely there is a causal association.

Clinical and animal studies suggest that Probucol may reduce AD risk and attenuate amyloidosis [26, 27, 39]. Suggested mechanisms include enhanced cerebrovascular efflux of soluble Aβ and neuro-protection as a consequence of suppression of oxidative pathways. Other indirect lines of evidence suggest that Probucol could confer AD protection by reducing vascular exposure to cytotoxic compounds including exaggerated plasma cholesterol, fatty acids or a reduction in inflammatory proteins including Aβ. The findings of this study support the latter notion and show that Probucol appears to suppress dietary fat induced stimulation of Aβ biosynthesis.

The 1 month dietary intervention study described in this study did not identify any significant increase in secretion of apo B lipoprotein-Aβ per se, and hence may reflect a localised phenomenon that is not particularly relevant to AD risk. Clearly, longer term feeding studies with an emphasis on the effects of Probucol on blood–brain barrier integrity and plasma Aβ homeostasis are warranted.

Acknowledgments The National Health and Medical Research Council of Australia, and the Australian Technology Network Centre supported this research financially.

Conflict of interest The authors have no conflicts of interest to declare in relation to this article.

References

1. Pastorino L, Lu KP (2006) Pathogenic mechanisms in Alzheimer's disease. Eur J Pharmacol 545:29–38
2. Selkoe DJ (1994) Cell biology of the amyloid beta-protein precursor and the mechanism of Alzheimer's disease. Annu Rev Cell Biol 10:373–403
3. Kapeller R, Cantley L (1994) Phosphatidylinositol 3-kinase. BioEssays 16:565–576
4. Luo Y, Hirashima N, Li Y, Alkon D, Sunderland T, Etcheberrigaray R, Wolozin B (1995) Physiological levels of β-amyloid increase tyrosine phosphorylation and cytosolic calcium. Brain Res 681:65–74
5. Luo Y, Sunderland T, Roth GS, Wolozin B (1996) Physiological levels of β-amyloid peptide promote PC12 cell proliferation. Neurosci Lett 217:125–128
6. Jekabsone A, Mander P, Tickler A, Sharpe M, Brown G (2006) Fibrillar beta-amyloid peptide Abeta1–40 activates microglial proliferation via stimulating TNF-alpha release and H_2O_2 derived from NADPH oxidase: a cell culture study. J Neuroinflamm 3:24
7. Sondag C, Dhawan G, Combs C (2009) Beta amyloid oligomers and fibrils stimulate differential activation of primary microglia. J Neuroinflamm 6:1
8. Haass C, Koo EH, Mellon A, Hung AY, Selkoe DJ (1992) Targeting of cell-surface beta-amyloid precursor protein to lysosomes-alternative processing into amyloid-bearing fragments. Nature 357:500–503
9. Panza F, Solfrizzi V, D'Introno A, Capurso C, Colacicco A, Torres F, Altomare E, Capurso A (2002) Genetics of late-onset Alzheimer's disease: vascular risk and beta-amyloid metabolism. Recenti Prog Med 93:489–497
10. Rocchi A, Orsucci D, Tognoni G, Ceravolo R, Siciliano G (2009) The role of vascular factors in late-onset sporadic Alzheimer's disease. Genetic and molecular aspects. Curr Alzheimer Res 6:224–237
11. Crossgrove J, Li G, Zheng W (2005) The choroid plexus removes beta-amyloid from brain cerebrospinal fluid. Exp Biol Med (Maywood) 230:771–776
12. Deane R, Sagare A, Hamm K, Parisi M, LaRue B, Guo H, Wu Z, Holtzman D, Zlokovic B (2005) IgG-assisted age-dependent clearance of Alzheimer's amyloid beta peptide by the blood-brain barrier neonatal Fc receptor. J Neurosci 25:11495–11503
13. Mamo JCL, Jian L, James AP, Flicker L, Esselmann H, Wiltfang J (2008) Plasma lipoprotein β-amyloid in subjects with Alzheimer's disease or mild cognitive impairment. Ann Clin Biochem 45:395–403
14. Burges B, McIsaac S, Naus K, Chan J, Tansley G, Yang J, Miao F, Ross C, van Eck M, Hayden M, van Nostrand W, St George-Hyslop P, Westaway D, Wellington C (2006) Elevated plasma triglyceride levels precede amyloid deposition in Alzheimer's disease mouse models with abundant A beta in plasma. Neurobiol Dis 24:114–127
15. Namba Y, Tsuchiya H, Ikeda K (1992) Apolipoprotein B immunoreactivity in senile plaque and vascular amyloids and neurofibrillary tangles in the brains of patients with Alzheimer's disease. Neurosci Lett 134:264–266
16. Takechi R, Galloway S, Pallebage-Gamarallage MMS, Wellington CL, Johnsen RD, Dhaliwal SS, Mamo JCL (2010) Differential effects of dietary fatty acids on the cerebral distribution of plasma-derived apo B lipoproteins with amyloid-β. Br J Nutr 103:652–662
17. Funatsu T, Suzuki K, Goto M, Arai Y, Kakuta H, Tanaka H, Yasuda S, Ida M, Nishijima S, Miyata K (2001) Prolonged inhibition of cholesterol synthesis by atorvastatin inhibits apo B-100 and triglyceride secretion from HepG2 cells. Atherosclerosis 157:107–115
18. Watts GF, Chan DC, Barrett PHR, O'Neill FH, Thompson GR (2003) Effect of a statin on hepatic apolipoprotein B-100 secretion and plasma campesterol levels in the metabolic syndrome. Int J Obes Relat Metab Disord 27:862–865
19. Pal S, Allister E, Thomson A, Mamo JCL (2002) Cholesterol esters regulate $apoB_{48}$ secretion in $CaCo_2$ cells. Atherosclerosis 161:55–63
20. Staels B, Dallongeville J, Auwerx J, Schoonjans K, Leitersdorf E, Fruchart J-C (1998) Mechanism of action of fibrates on lipid and lipoprotein metabolism. Circulation 98:2088–2093
21. Dufouil C, Richard F, Fievet N, Dartigues J, Ritchie K, Tzourio C, Amouyel P, Alperovitch A (2005) APOE genotype, cholesterol level, lipid-lowering treatment, and dementia: the three-city study. Neurology 64:1531–1538
22. Rockwood K, Kirkland S, Hogan D, MacKnight C, Merry H, Verreault R, Wolfson C, McDowell I (2002) Use of lipid-lowering agents, indication bias, and the risk of dementia in community-dwelling elderly people. Arch Neurol 59:223–227
23. Mamo J, Elsegood C, Umeda Y, Hirano T, Redgrave T (1993) Effect of probucol on plasma clearance and organ uptake of chylomicrons and VLDLs in normal and diabetic rats. Arterioscler Thromb 13:231–239
24. Mellies MJ, Gartside PS, Glatfelter L, Vink P, Guy G, Schonfeld G, Glueck CJ (1980) Effects of probucol on plasma cholesterol, high and low density lipoprotein cholesterol, and apolipoproteins A1 and A2 in adults with primary familial hypercholesterolemia. Metabolism 29:956–964

25. Gershkovich P, Hoffman A (2005) Uptake of lipophilic drugs by plasma derived isolated chylomicrons: linear correlation with intestinal lymphatic bioavailability. Eur J Pharm Sci 26:394–404
26. Poirier J (2003) Apolipoprotein E and cholesterol metabolism in the pathogenesis and treatment of Alzheimer's disease. Trends Mol Med 9:94–101
27. Poirier J (2005) Apolipoprotein E, cholesterol transport and synthesis in sporadic Alzheimer's disease. Neurobiol Aging 26:355–361
28. Galloway S, Jian L, Johnsen R, Chew S, Mamo JCL (2007) β-Amyloid or its precursor protein is found in epithelial cells of the small intestine and is stimulated by high-fat feeding. J Nutr Biochem 18:279–284
29. Pallebage-Gamarallage MM, Galloway S, Johnsen R, Jian L, Dhaliwal S, Mamo JCL (2009) The effect of exogenous cholesterol and lipid-modulating agents on enterocytic amyloid-β abundance. Br J Nutr 101:340–347
30. Galloway S, Pallebage-Gamarallage MM, Takechi R, Jian L, Johnsen RD, Dhaliwal SS, Mamo JC (2008) Synergistic effects of high fat feeding and apolipoprotein E deletion on enterocytic amyloid-beta abundance. Lipids Health Dis 22:7–15
31. Takechi R, Galloway S, Pallebage-Gamarallage M, Johnsen R, Mamo J (2008) Three-dimensional immunofluorescent double labelling using polyclonal antibodies derived from the same species: enterocytic colocalization of chylomicrons with Golgi apparatus. Histochem Cell Biol 129:779–784
32. Lairon D (2008) Macronutrient intake and modulation on chylomicron production and clearance. Atheroscler Suppl 9:45–48
33. Galloway S, Takechi R, Pallebage-Gamarallage M, Dhaliwal S, Mamo J (2009) Amyloid-beta colocalizes with apolipoprotein B in absorptive cells of the small intestine. Lipids Health Dis 8:46
34. Smith D, Watts GF, Dane-Stewart C, Mamo JC (1999) Postprandial chylomicron response may be predicted by a single measurement of plasma apolipoprotein B48 in the fasting state. Eur J Clin Invest 29:204–209
35. Ohtani H, Hayashi K, Hirata Y, Dojo S, Nakashima K, Nishio E, Kurushima H, Saeki M, Kajiyama G (1990) Effects of dietary cholesterol and fatty acids on plasma cholesterol level and hepatic lipoprotein metabolism. J Lipid Res 31:1413–1422
36. Sharon A, Michael IM, Paul ND (2000) The effects of fatty acids on apolipoprotein B secretion by human hepatoma cells (HEP G2). Atherosclerosis 150:255–264
37. Runz H, Rietdorf J, Tomic I, de Bernard M, Beyreuther K, Pepperkok R, Hartmann T (2002) Inhibition of intracellular cholesterol transport alters presenilin localisation and amyloid precursor protein processing in neuronal cells. J Neurosci 22: 1679–1689
38. Tawara K, Tomikawa M, Abiko Y (1986) Mode of action of probucol in reducing serum cholesterol in mice. Jpn J Pharmacol 40:123–133
39. Champagne D, Pearson D, Dea D, Rochford J, Poirier J (2003) The cholesterol-lowering drug probucol increases apolipoprotein E production in the hippocampus of aged rats: implications for Alzheimer's disease. Neuroscience 121:99–110

Article 4:

Takechi R, **Galloway S**, Pallebage-Gamarallage MM, Lam V, Mamo JCL. (2009) Dietary fats, cerebrovascular integrity and Alzheimer's disease risk. *Prog Lipid Res*. 49, 159-170

Synopsis:

This study addresses the latter part of the broad hypothesis and examines the putative effects of saturated and unsaturated feeding on BBB integrity. The primary finding of chapter 5 was that enterocytic Aβ is enhanced under high-fat feeding. Secretion of plasma Aβ-lipoproteins from enterocytes may not be transported into the brain without demonstrable BBB damage and thus increased "leakage" and contribution to pathology of AD was questionable. This study directly extends on the findings of chapter 5 and demonstrates that saturated fat diets, not MUFA or PUFA, causes damage to BBB. I contributed to care of animals, design of dietary intervention, sample collection and sample analysis.

Background:

Alzheimer's disease patients exhibit cerebrovascular disturbances such as sequestration of Aβ within the blood vessels of the brain, proliferation of vascular cells (Ellis et al. 1996) and inflammation (Cullen 1997, Itagaki et al. 1989). Moreover, increases in identification of plasma-derived proteins are found in AD brains (Kalaria 1992, Wisniewski et al. 1997b). These observations are consistent with the notion of damaged BBB and underlying manifestation of vascular pathology in AD. Increases in plasma Aβ transport to the brain coupled with inadequate clearance of Aβ from brain (Donahue et al. 2006) can cause accumulation of Aβ and a pathological cascade of events leading to synaptic toxicity and neuronal cell death.

Plasma Aβ can be derived from lipogenic organs and the liver and small intestines can be a significant source of Aβ (Galloway et al. 2007, Takechi et al. 2008a, Koudinov and Koudinova 1997). Hepatocytes and enterocytes secrete Aβ in complex with lipoproteins and in the liver and small intestine, respectively. We

previously found Aβ production is regulated by intake of dietary fats (Galloway et al. 2007, Koudinov and Koudinova 1997, James et al. 2003). Consistent in the literature, SFA intake significantly increases enterocytic abundance of Aβ which associates with enterocytic lipoprotein-apo B prior to secretion into plasma. Increases in postprandial lipoproteins derived from enterocytes were previously observed in cognitively impaired and AD patients compared to age-matched controls (Mamo et al. 2008).

The relationship between vascular disturbances present in AD and dietary induced increases in enterocytic and plasma Aβ lead to the current study examining the role of dietary fats in maintenance of BBB integrity.

Methods:

Female C57BL6/J mice were fed HF diet (20.3 % w/w as fat) containing predominantly SFA, PUFA or MUFA fats and a LF (4 % w/w as fat) control diet for 3 and 6 months. Plasma Aβ and S100β was analysed using ELISA methods as per manufacturer's instructions. Cerebral cryosections were immunostained for IgG, apo B, occludin and von Willebrand factor (vWF) and signal was determined using semi-quantitative immunofluorescent microscopy. Co-localisation of perivascular Aβ with apo B and occludin with vWF were determined by dual-labelling using two polyclonal antibodies as previously described (Takechi et al. 2008b). Co-localisation of apo B and amyloid plaques were also determined in separately provided APP/PS1 brain tissue using simular methods of analysis and detection.

Results:

Extravasation of plasma IgG and apo B was significantly increased in the cerebral cortex and brain stem of SFA fed mice compared to other groups (Figure 2). This increase in IgG and apo B was also observed in transgenic mice in the same brain regions as well as the hippocampus (Figure 5). Co-localization of perivascular Aβ with apo B was present in-situ indicating the transport of Aβ-apo B occurring as a result of SFA diet (Figure 3). Blood-brain-barrier integrity was assessed by measurement of occludin relative to vWF, which was significantly reduced in SFA fed diet and in APP/PS1 mice. In addition, plasma S100β, the

marker of brain to blood efflux, was significantly increased in SFA fed mice (>80%) and doubled in APP/PS1 mice.

Discussion:

Both influx and efflux functions of BBB were shown to be exacerbated under SFA feeding and in APP/PS1 mice. Compared to MUFA and PUFA groups, SFA feeding for 3 months was sufficient to induce cell junction changes within BBB and increased extravasation of plasma proteins, IgG and apo B into the brain. In addition, colocalization of Aβ and apo B within cerebral blood vessels and amyloid plaques suggests that intestinally or hepatically derived Aβ-apo B-lipoproteins can cross the BBB. Diets were well tolerated and there was no difference in weight gain, plasma triglycerides or plasma cholesterol indicating no pleiotropic effects of diets. Changes physiological function of BBB can therefore be regarded as a direct result of SFA feeding. Although the direct modes of action of SFA are currently unclear, several plausible mechanisms exist. Blood-brain barrier can be compromised via vasoactive properties of the plasma Aβ-lipoprotein entity alone or via induction of reactive oxygen species, changes in intracellular calcium homeostasis, increased stress on the endoplasmic reticulum (Morgan 2009, Carpentier 2008, Cnop et al. 2008, Solfrizzi et al. 2010) or broader toxic effects of dietary fatty acids on BBB. This study extends previous findings and provides new insight in relation to mechanisms surrounding SFA diet induced increases to AD risk. Reduced AD risk associated with MUFA or n-3 PUFA intake is also supported in this study.

British Journal of Nutrition (2010), **103**, 652–662
doi:10.1017/S0007114509992194

Differential effects of dietary fatty acids on the cerebral distribution of plasma-derived apo B lipoproteins with amyloid-β

Ryusuke Takechi[1,2,3], Susan Galloway[1,2,3], Menuka M. S. Pallebage-Gamarallage[1,2,3], Cheryl L. Wellington[4], Russell D. Johnsen[5,6], Satvinder S. Dhaliwal[1,2,3] and John C. L. Mamo[1,2,3]*

[1]*Faculty of Health Science, School of Public Health, Curtin University of Technology, Bentley, WA, Australia*
[2]*The Curtin Health Innovation Research Institute, Bentley, WA, Australia*
[3]*The Australian Technology Network, Centre for Metabolic Fitness, Perth, WA, Australia*
[4]*Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada*
[5]*Centre for Neuromuscular and Neurological Disorders, University of Western Australia, Nedlands, WA, Australia*
[6]*The Australian Neuromuscular Research Institute, Nedlands, WA, Australia*

(Received 5 June 2009 – Revised 26 August 2009 – Accepted 27 August 2009 – First published online 28 October 2009)

Some dietary fats are a risk factor for Alzheimer's disease (AD) but the mechanisms for this association are presently unknown. In the present study we showed in wild-type mice that chronic ingestion of SFA results in blood–brain barrier (BBB) dysfunction and significant delivery into the brain of plasma proteins, including apo B lipoproteins that are endogenously enriched in amyloid-β (Aβ). Conversely, the plasma concentration of S100B was used as a marker of brain-to-blood leakage and was found to be increased two-fold because of SFA feeding. Consistent with a deterioration in BBB integrity in SFA-fed mice was a diminished cerebrovascular expression of occludin, an endothelial tight junction protein. In contrast to SFA-fed mice, chronic ingestion of MUFA or PUFA had no detrimental effect on BBB integrity. Utilising highly sensitive three-dimensional immunomicroscopy, we also showed that the cerebral distribution and co-localisation of Aβ with apo B lipoproteins in SFA-fed mice are similar to those found in amyloid precursor protein/presenilin-1 (APP/PS1) amyloid transgenic mice, an established murine model of AD. Moreover, there was a strong positive association of plasma-derived apo B lipoproteins with cerebral Aβ deposits. Collectively, the findings of the present study provide a plausible explanation of how dietary fats may influence AD risk. Ingestion of SFA could enhance peripheral delivery to the brain of circulating lipoprotein–Aβ and exacerbate the amyloidogenic cascade.

Alzheimer's disease: Amyloid-β: Blood–brain barrier: SFA: TAG-rich lipoproteins

An accumulating body of evidence is consistent with the concept that the onset and progression of Alzheimer's disease (AD) is influenced by lifestyle factors including nutrition[1]. Several population studies have found that SFA are a positive risk factor for AD[2–3] and in animal models of AD, SFA or cholesterol feeding markedly exacerbates cerebral pathology[4–5]. However, the mechanisms that link dietary fat to the pathogenesis of AD are unclear.

The cerebrovasculature in subjects with AD shows pathological alterations including vascular endothelial and smooth muscle cell proliferation[6]. Blood plasma proteins have been detected in the parenchyma of AD brains[7–8] and inflammatory sequalae are commonly reported[9–10], observations that are consistent with breakdown of the blood–brain barrier (BBB). Yet despite evidence supportive of AD having an underlying vascular component, most research focuses on damage of neurons[11].

A major neuropathological marker of AD is amyloid-β (Aβ) deposition in the cerebrovasculature and in the cerebral parenchyma. Derived from amyloid precursor protein (APP), Aβ is the predominant component of amyloid plaque[12]. The source of cerebral Aβ deposits in AD is uncertain, though there is little evidence for increased cerebral Aβ production in sporadic, late-onset AD which accounts for over 96 % of AD cases. Rather, decreased Aβ clearance across the BBB via receptor pathways and/or via the choroid plexus has been suggested as an initiating pathway for amyloidosis[13–15]. More recent has been evidence of blood-to-brain delivery of circulating Aβ, a process that would conceivably exacerbate parenchymal load in the absence of compensatory clearance pathways[16].

Plasma Aβ can be derived from vascular smooth muscle cells and endothelial cells, or from blood platelets. However, another significant source of plasma Aβ may be from lipogenic organs such as the small intestine and liver[17–19]. Hepatocytes and absorptive epithelial cells of the small intestine (enterocytes) secrete Aβ as a lipoprotein complex, and in the small intestine this pathway is under dietary regulation[17–20].

Abbreviations: Aβ, amyloid-β; AD, Alzheimer's disease; APP, amyloid precursor protein; BBB, blood–brain barrier; BS, brain stem; CTX, cortex, excluding the hippocampus; 3-D, three-dimensional; HPF, hippocampal formation; PS1, presenilin-1; TRL, TAG-rich lipoprotein; vWF, von-Willebrand factor.

* **Corresponding author:** Professor John Mamo, fax +61 8 92662958, email J.Mamo@Curtin.edu.au

Chronic ingestion of SFA was reported to significantly enhance enterocytic abundance of Aβ and conversely the protein could not be detected when animals were fasted[17].

Distributional analysis of plasma lipoprotein–Aβ in normal subjects and those with AD or mild cognitive impairment (MCI) is consistent with the notion that dietary-induced Aβ may contribute to the aetiology of AD[21]. In control and in AD/MCI subjects, greater than 60 % of plasma lipoprotein–Aβ was found to be associated with TAG-rich lipoproteins (TRL); however, this was significantly increased in AD/MCI subjects *v.* controls. Moreover, whilst the AD subjects were normolipidaemic and had similar plasma levels of hepatically derived lipoproteins, the concentration of apo B48 (an exclusive marker of chylomicrons) was elevated more than three-fold in post-absorptive AD subjects. Increased apo B48 is indicative of postprandial dyslipidaemia, an exaggerated rise in plasma chylomicrons that occurs following the ingestion of dietary fats[22]. Consistent with the notion of an Aβ postprandial response, ingestion of a lipid-enriched meal was found to cause a transient increase in the plasma concentration of APP and of Aβ in otherwise healthy subjects[23].

Evidence of a causal link between plasma lipoprotein–Aβ and AD also comes from studies in animal models of AD. In transgenic mice that over-express APP, plasma Aβ concentration correlated with secretion rates into the blood of TRL, which was increased 3- to 8-fold above wild-type controls[24]. Furthermore, there was a positive association between plasma TRL–Aβ secretion with onset of cerebrovascular and parenchymal amyloidosis[25].

The mechanisms by which circulating Aβ increases AD risk are presently unclear. However, several studies have provided evidence of a vasoactive role of Aβ, with pathological manifestations before Aβ deposition[26–28]. Aβ is vasoconstrictive and vessels treated with Aβ show significant endothelial cell damage, with changes in the cell membrane, cytoplasm, nucleus and other organelles.

We hypothesise that SFA may adversely influence BBB function, because of exaggerated exposure to plasma lipoprotein–Aβ that leads to greater rates of plasma to cerebral Aβ delivery. In previous studies, integrity of the BBB has been assessed in experimental colitis[29] and following acute intravenous injection of solubilised, exogenous Aβ[26–28]. Consistent with the notion that lipoproteins have an important role in central nervous system diseases, Kay *et al.* reported significant remodelling of cerebrospinal fluid lipoproteins after subarachnoid haemorrhage[30]. However, the potential effect of SFA on plasma Aβ homeostasis and BBB function have not been reported. If this hypothesis is correct, it may explain epidemiological data that link SFA intake with AD risk. In the present study we directly explore BBB integrity in wild-type mice fed fatty acid-enriched diets.

Materials and methods

Animals

Female C57BL/6J mice, aged 6 weeks, were purchased from the Animal Resource Centre (Perth, WA, Australia). Mice were randomly allocated to either the control or one of three fatty acid treatment groups (see Dietary intervention in wild-type mice section). Mice were maintained in an accredited animal holding facility with regulated temperature, air pressure and lighting (12 h light–12 h dark). Mice had *ad libitum* access to feed and water. At 3 and 6 months following commencement of the dietary intervention, six mice from each group were killed by cardiac exsanguination under complete anaesthesia. The C57BL6J mice were considered an appropriate wild-type strain because the transgenic APP/presenilin-1 (APP/PS1) mice are a C57BL6J × C3H strain.

Double transgenic APP/PS1 mice develop AD-like brain pathology after 20 weeks of age. In the present study APP/PS1 mice were fed a standard low-fat rodent chow and killed at 12 months of age.

All experimental procedures used in this project were approved by a National Health and Medical Research Council (Australia) accredited Animal Ethics Committee (Curtin University approval no. R34/08).

Dietary intervention in wild-type mice

The feed preparations were made by Glenn Forest Stock Feeders (Perth, WA, Australia). The low-fat control diet was a standard American Institute of Nutrition AIN-93M rodent chow containing < 4 % (w/w) fat as polyunsaturates, with < 1 % total digestible energy as lipids and was free of cholesterol (for details, see Table 1). All of the fat-enriched diets comprised 40 % total digestible energy as lipids (or 20·3 %, w/w). For the SFA intervention group, the principal fatty acid types were palmitic (16 : 0) and stearic (18 : 0) (13 %, w/w) but the SFA diet also contained some oleic acid (18 : 1*n*-9, 6 %, w/w). The MUFA diet contained approximately 16 % as oleic acids, approximately 1·5 % as SFA and only trace amounts of PUFA. The PUFA-enriched diet was principally made up of DHA (22 : 6*n*-3), EPA (20 : 5*n*-3) and oleic acid. SFA and MUFA accounted for less than 4 % (w/w) of the PUFA-enriched diet. The MUFA and PUFA diets reflected blends of Sunola™ oil and fish oils, respectively.

Tissue and plasma sample collection

Following dietary intervention for 3 or 6 months, mice were anaesthetised with pentobarbitone and blood samples obtained by cardiac puncture. Plasma was separated by low-speed centrifugation and stored immediately at −80°C. Brains were carefully removed and washed in chilled PBS. For immunofluorescent microscopy, the right hemisphere was segmented and fixed in 4 % paraformaldehyde for 24 h followed by cryoprotection in 20 % sucrose solution for 3 d at 4°C. Tissues were then frozen in isopentane/dry ice and stored at −80°C.

Plasma S100B and amyloid-β analysis

Plasma S100B was measured by ELISA (CosmoBio, Tokyo, Japan) according to the instructions provided by the manufacturer. Briefly, 20 μl of plasma samples, or of the S100B standards (0, 98, 197, 394, 1575, 3150 and 6300 pg/ml) were incubated overnight at 4°C in ninety-six-well microplates coated with the primary antibody. Thereafter, plates were incubated with the conjugated secondary

Table 1. Dietary composition data sheet*
(Percentages)

Diet...	Control	SFA	MUFA	PUFA
Total fat	4	20·3	20·3	20·3
Total digestible energy from lipids	n/a	40	40	40
SFA, 12 : 0 and less	n/a	n/a	Not detected	n/a
Myristic acid (14 : 0)	Trace	0·05	0·02	0·54
Pentadecanoic acid (15 : 0)	n/a	0·01	n/a	0·16
Palmitic acid (16 : 0)	0·20	5·16	0·85	3·26
Magaric acid (17 : 0)	n/a	0·05	n/a	0·18
Stearic acid (18 : 0)	0·10	7·31	0·87	0·92
Arachidic acid (20 : 0)	n/a	0·24	n/a	0·06
Behenic acid (22 : 0)	n/a	0·04	n/a	n/a
Tetracosanoic acid (24 : 0)	n/a	0·03	n/a	n/a
Palmitoleic acid (16 : 1)	Trace	0·05	0·02	0·66
Heptadecenoic acid (17 : 1)	n/a	0·01	n/a	0·10
Oleic acid (18 : 1*n*-9)	2·40	6·62	15·70	2·25
Gadoleic acid (20 : 1)	Trace	0·01	0·07	0·18
Linoleic acid (18 : 2*n*-6)	0·80	0·67	2·42	0·23
α-Linolenic acid (18 : 3*n*-3)	n/a	0·05	0·13	0·09
γ-Linolenic acid (18 : 3*n*-6)	0·40	Not detected	n/a	0·08
Stearidonic acid (18 : 4*n*-3)	n/a	n/a	0·08	n/a
Arachidonic acid (20 : 4*n*-6)	Trace	Not detected	0·20	0·46
EPA (20 : 5*n*-3)	Trace	Not detected	Not detected	2·00
DPA (22 : 5*n*-3)	n/a	Not detected	Not detected	0·30
DHA (22 : 6*n*-3)	Trace	Not detected	Not detected	8·22

n/a, Not applicable.
* Detailed dietary compositions of the diet of low-fat control, saturated fat (SFA), monounsaturated fat (MUFA) and polyunsaturated fat (PUFA) groups are given.

antibody for 2 h, followed by 2 h incubation with streptavidin–horseradish peroxidase. Finally samples were incubated with substrate solution for 20 min and the reaction was terminated with stopping solution. The optical absorbance was measured at 490 nm.

Plasma concentrations of mouse $A\beta_{1-40}$ and $A\beta_{1-42}$ were measured utilising Biosource ELISA kits (KMB3441; Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Plasma (100 μl) or Aβ standards ($A\beta_{1-40}$: 0, 7·8, 15·6, 31·3, 62·5, 125, 250, 500 pg/ml; $A\beta_{1-42}$: 0, 3·12, 6·25, 12·5, 25, 50, 100, 200 pg/ml) were dispensed into wells and incubated for 2 h at room temperature, then thoroughly washed. The primary antibody for either $A\beta_{1-40}$ or $A\beta_{1-42}$ was then added and incubated for 1 h at room temperature. Anti-rabbit IgG–horseradish peroxidase was added for 30 min, then incubated with stabilised chromogen for an additional 30 min in darkness. The optical absorbance was measured at 450 nm.

Immunofluorescent detection of IgG, apo B, occludin and von Willebrand factor in cerebral tissue

Cryosection specimens of 18 μm were prepared from the right hemisphere of the brain of each mouse. For IgG detection, sections were incubated with polyclonal goat anti-mouse IgG antibody conjugated with Alexa 488 fluorochrome (1:100; Abcam, Inc., Cambridge, MA, USA) for 2 h at room temperature. Subsequently, the sections were imaged utilising an inverted fluorescent microscope (Zeiss AxioVert 200M; Carl Zeiss, Jena, Germany) and AxioVision software (version 4.6; Carl Zeiss).

Apo B lipoproteins were detected by overnight incubation with polyclonal rabbit anti-apo B antibody (1:200; Abcam, Inc.). The primary antibody was then visualised with goat-anti-rabbit IgG conjugated with Alexa 488 (Invitrogen).

Negative controls were included for all immunofluorescent experiments and included replacement of the primary antibody with buffer, or an irrelevant serum. Fluorescent staining was not observed for any negative control tissue preparations.

Fluorescent image capture and semi-quantitative measurement

All fluorescent images were captured with a fluorescent microscope (AxioVert 200M) coupled to an MRm digital camera and managed by AxioVision software (version 4.6; Carl Zeiss). Three-dimensional (3-D) images were taken with ApoTome optical sectioning methodology (Carl Zeiss). Quantification was determined within the cortex excluding the hippocampus (CTX), hippocampal formation (HPF) and brain stem (BS).

For each mouse killed, a minimum of three cryosection specimens was prepared from the right hemisphere of the brain. For each specimen, up to seven 3-D ApoTome images were randomly taken within each designated region of the brain. For IgG and apo B quantitative measurement, images were captured at × 200 magnification (430 × 322 μm). Each 3-D image consisted of six to thirteen Z-stack images and the distance between Z-stack slices was 1·225 μm optimised by Nyquist theory (2 × oversampling in axial direction). The optical densitometric sum for the protein of interest was determined in three dimensions (1388 × 1040 pixel two-dimensional planes) utilising the automated optical density measurement tool (AxioVision; Carl Zeiss).

Double immunofluorescent detection with two polyclonal antibodies

In order to detect two different proteins with two polyclonal antibodies raised in the same species, a double immunofluorolabelling method was used as we have previously described[31]. For the co-localisation of Aβ with perivascular leakage of plasma apo B, rabbit polyclonal anti-$A\beta_{1-40/42}$ (Chemicon; Millipore, Billerica, MA, USA) and rabbit polyclonal anti-apo B antibodies were used. For the BBB tight junction protein occludin and the vascular endothelial cell marker von Willebrand factor (vWF), these were detected with rabbit polyclonal anti-occludin-1 (Invitrogen) and rabbit polyclonal anti-vWF antibodies (Abcam, Inc.). Briefly, 10 μm sections were fixed in acetone for 3 min at −20°C and heat-mediated antigen retrieval was achieved by incubating in a water-bath at 60°C for 3 h. Endogenous biotin was blocked with avidin in egg white and biotin in skimmed milk. In order to avoid the cross-reaction of the two polyclonal antibodies, the concentration of the first antibody was diluted so that it was undetectable with conventional secondary antibody detection, but detectable after the signal amplification with the biotin–avidin reaction. Subsequently the second protein of interest was detected with standard secondary detection.

For the co-localisation analysis of Aβ and apo B in cerebral tissue of wild-type mice given different fatty acid-enriched diets, fifteen 3-D images were captured at × 400 magnification (222 × 166 μm). Each of the 3-D images contained thirty-two to seventy-one Z-stack images and the distance between Z-stack slices was 0·275 μm. From a total of 695 two-dimensional images, co-localisation of Aβ and apo B was then determined with AxioVision software and data were expressed as Manders' and Pearson's correlation coefficients. Manders' correlation coefficient estimates the co-localisation independent of fluorescent intensity, whereas Pearson's correlation coefficient explores if there is also an association in fluorescent intensities.

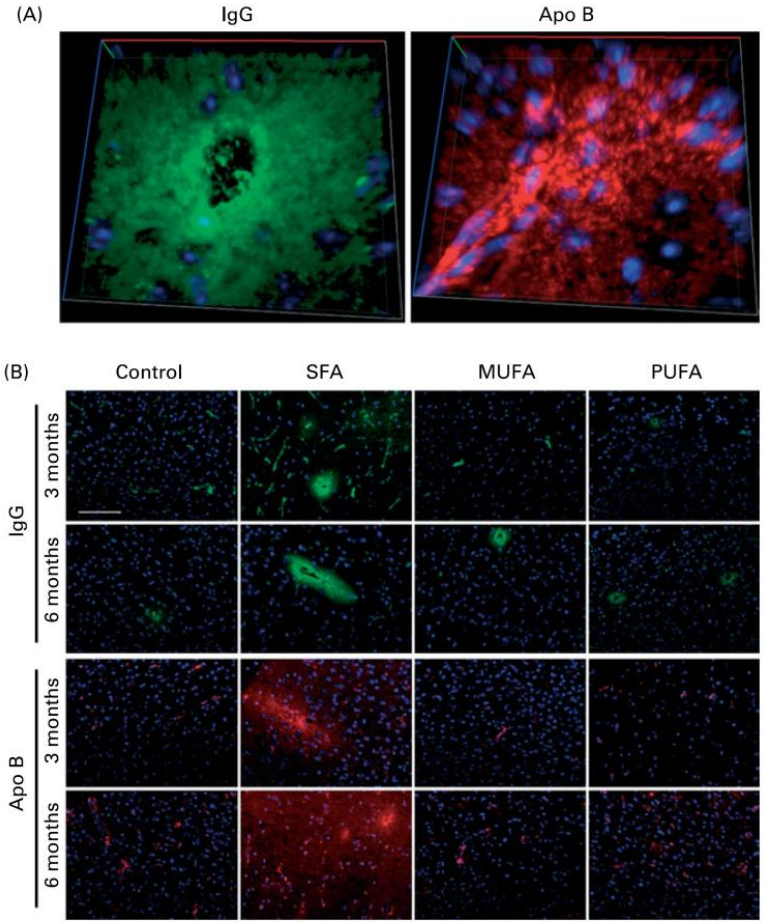


Fig. 1. Three-dimensional (3-D) immunodetection of cerebral IgG and apo B extravasation. Perivascular leakage of plasma IgG and apo B into the brain was captured in 3-D utilising highly sensitive immunofluorescent microscopy techniques. IgG is shown in green and apo B is in red. 6-Diamidino-2-phenylindole (DAPI)-counterstained nuclei are shown in blue. (A) Representative 3-D images of plasma IgG and apo B leakage observed in mice fed a SFA-rich diet for 6 months. Scales of *x* (red), *y* (red) and *z* (green) axes are 90 × 80 × 10 μm. (B) Low magnification images of cerebral IgG and apo B immunoreactivity. Significant cerebral extravasation of IgG and apo B was seen in both 3 and 6 month SFA groups. The scale bar indicates 100 μm.

For vWF and occludin measurement, 3-D ApoTome images were taken at × 200 magnification. Each 3-D image consisted of a minimum of three and up to seven Z-stack images and distance between Z-stack slices was 1·225 μm. To measure the relative abundance of vWF, the optical densitometric sum within the image was determined and expressed as per vWF per volume of tissue. In order to measure occludin abundance specific for the cerebrovasculature, only occludin staining which co-localised with vWF was measured.

Co-localisation of apo B with cerebral amyloid plaques

To examine the co-localisation of apo B with amyloid plaques in 12-month-old APP/PS1 transgenic mice, the immunofluorescent double labelling method was used as described for the wild-type mice given fat-enriched diets. In the APP/PS1 mice, 50 μm thick cryosections were fixed with 4 % paraformaldehyde for 1 h and heat-mediated antigen retrieval was done by incubating the sections in 60°C water for 3 h. A mixture of rabbit-derived anti-apo B and mouse monoclonal anti-Aβ antibodies was applied and incubated for 3 d at 4°C. Following washing with PBS, the primary antibodies were detected with anti-rabbit IgG Alexa 488 and anti-mouse IgG1 Alexa 680, respectively. Immunofluorescent 3-D images were captured at × 400 magnification with ApoTome.

Statistical analysis

There were twelve mice in each dietary group studied. For the immunodetection of IgG, apo B, occludin and vWF, up to seven 3-D images were obtained for each of the three regions of the brain studied (CTX, HPF and BS). Each 3-D image was generated from a stack of two-dimensional images, consisting of between twenty-two and 181 sequential images. The 3-D stacks represented 4·9–18·5 μm tissue thickness. For co-localisation analysis of apo B with Aβ in SFA-fed mice and in APP/PS1 mice, three to six 3-D images were generated per animal, with ten mice in the SFA group and six mice in the APP/PS1 group. A total of 694 images were analysed in SFA-fed mice and 834 images in transgenic APP/PS1 mice. Data were normally distributed and compared by one-way ANOVA followed by Tukey's *post hoc* test or Student's *t* test using SPSS (SPSS, Inc., Chicago, IL, USA). Data were expressed as mean values with their standard errors.

Results

The fatty acid-supplemented diets given to mice were well tolerated. There was no difference in plasma lipids or body weight following 3 or 6 months of dietary intervention (data not shown).

IgG (150 kDa) immunoreactivity was used as a generic marker of blood-to-brain influx of plasma proteins. In mice fed the SFA diet for 3 months, we identified significant perivascular leakage of IgG into the CTX, BS and to a lesser extent, within the HPF (Figs. 1 and 2). At 6 months of feeding, IgG leakage was more evident than at 3 months (Figs. 1 and 2). In contrast, in the mice given the low-fat control diet, or either of the unsaturated fatty acid diets, there was no evidence of cerebral IgG immunoreactivity (Fig. 1).

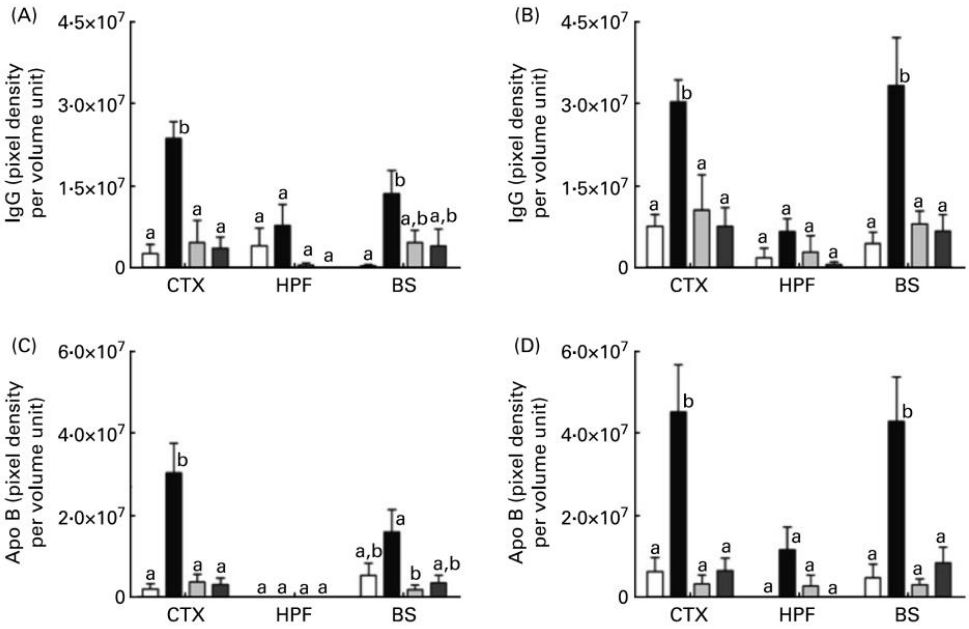


Fig. 2. Three-dimensional (3-D) semi-quantitative analysis of cerebral IgG (A and B) and apo B (C and D) extravasation in mice fed a control diet (□), a SFA-rich diet (■), a MUFA-rich diet (▩) or a PUFA-rich diet (■) for 3 months (A and C) or 6 months (B and D). The extent of cerebral IgG and apo B abundance was determined in 3-D based on the optical pixel density. Optical pixel densities were measured in three major brain regions of the cortex (CTX), hippocampal formation (HPF) and brain stem (BS) and expressed as per volume unit. Values are means, with standard errors represented by vertical bars. [a,b] Mean values, within a region, with unlike letters were significantly different ($P<0·05$; one-way ANOVA).

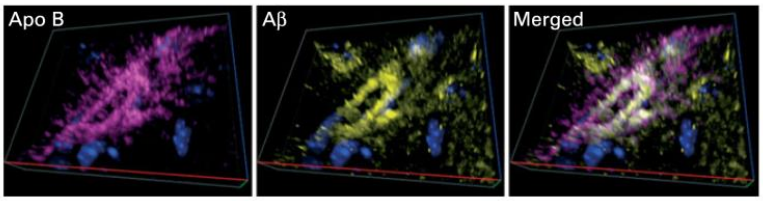


Fig. 3. Co-localisation of amyloid-β (Aβ) with perivascualr apo B influx in SFA-fed mice. Significant immunoreactivity of Aβ was detected concomitant with perivascular leakage of apo B lipoproteins in SFA-fed mice, consistent with blood-to-brain delivery of lipoprotein–Aβ. The immunofluorescent images were captured in three dimensions (x, y, $z = 70 \times 60 \times 11$ μm), and separated single images of apo B (magenta) and Aβ (yellow), and the merged image are shown. The number of co-localising pixels of different fluorescent dyes was measured by AxioVision software™ (Carl Zeiss, Jena, Germany) utilising Manders' correlation analysis (coefficient = 0·843 (SEM 0·01); n 695).

Apo B was used as a marker of intestinal and hepatically derived lipoprotein influx into the brain. Native apo B lipoproteins are large macromolecules of 3–50 million kDa molecular weight and a diameter of > 23 nm. In SFA-fed mice, apo B immunoreactivity was visible within the CTX and BS at 3 months following commencement of the diet (Fig. 1). However, ingestion of SFA for 6 months exacerbated apo B extravasation and apo B became more evident within the HPF. Analogous to the findings for IgG, mice maintained on the MUFA-enriched, PUFA-enriched or control diet showed no apo B cerebral immunoreactivity at either 3 or 6 months of feeding (Figs. 1 and 2).

Evidence consistent with the possibility that SFA feeding leads to peripheral delivery to the brain of apo B lipoprotein-associated Aβ is shown in Fig. 3. Perivascular distribution of Aβ is clearly seen coinciding with the distribution of apo B lipoproteins (Manders' correlation coefficient 0·843 (SEM 0·01); $P < 0·0001$).

To explore if the pattern of cerebral apo B/Aβ distribution in SFA mice could be relevant to AD aetiology, we also investigated IgG and apo B distribution in 12-month-old APP/PS1 transgenic animals. As found in SFA wild-type mice, the APP/PS1 mice have significant cerebral IgG and apo B extravasation primarily within the CTX, but with significant amounts also within the HPF (Figs. 4 and 5). In addition, utilising highly sensitive 3-D immunodetection, apo B lipoproteins were clearly visible co-localised with Aβ plaque (Manders' correlation coefficient 0·85 (SEM 0·004); $P < 0·0001$; Fig. 6) and plaque abundance positively correlated with apo B immunoreactivity (Pearson's correlation coefficient 0·49 (SEM 0·037)).

BBB integrity was also assessed by determining the abundance of occludin (a tight junction protein) relative to vWF. Both in SFA-fed mice and in APP/PS1 transgenic mice, occludin expression was substantially attenuated compared with controls (Figs. 7 and 8). Further evidence that BBB integrity was compromised was suggested by changes in the plasma concentration of S100B (Fig. 9). In the cerebrospinal fluid, S100B level is orders of magnitude greater than in plasma and therefore serves as a useful surrogate marker of

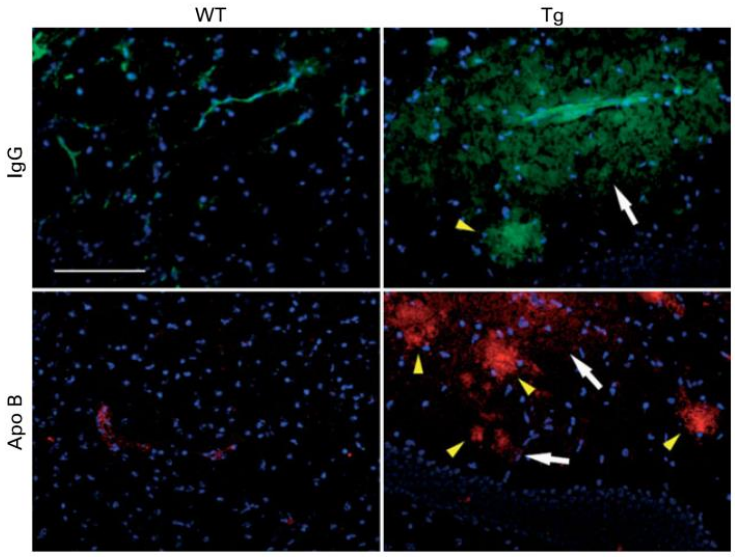


Fig. 4. Three-dimensional (3-D) quantitative immunomicroscopy of cerebral IgG and apo B extravasation in amyloid precursor protein/presenilin-1 (APP/PS1) amyloid transgenic (Tg) mice. The cerebral leakage of plasma IgG and apo B were quantitatively measured by 3-D immunofluorescent detection in the Tg mice. Substantial influx of plasma IgG and apo B into the brain was observed in the Tg mice (white arrows) while no leakages were seen in age-matched wild-type (WT) control mice. Notably, apo B immunoreactivity in the Tg mice strongly co-localised with amyloid plaques (see Fig. 6) while only occasional co-localisation of IgG with plaques was seen (yellow arrow heads). IgG, apo B and nuclei are shown in green, red and blue, respectively. The scale bar indicates 100 μm.

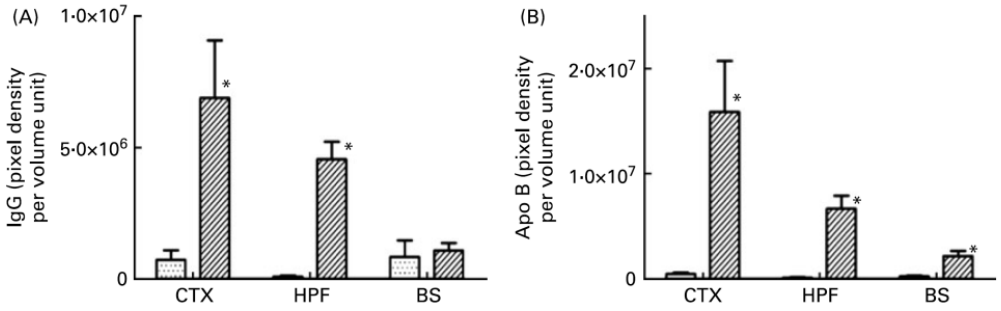


Fig. 5. Three-dimensional semi-quantitative analysis of cerebral IgG (A) and apo B (B) extravasation in amyloid precursor protein/presenilin-1 (APP/PS1) amyloid transgenic (Tg; ▨) mice. Optical pixel densities were measured in three major brain regions of the cortex (CTX), hippocampal formation (HPF) and brain stem (BS) and expressed as per volume unit. Values are means, with standard errors represented by vertical bars. *Quantitative analysis based on the pixel density suggested significantly increased immunoreactivity of IgG and apo B in the brain of Tg mice compared with wild-type control mice (⊠) ($P<0{\cdot}05$; *t* test).

brain-to-blood efflux[32–33]. Mice fed SFA had an 80 % increase in plasma S100B compared with low fat-fed mice, but there was no difference in mice fed either of the unsaturated fatty acid diets (Fig. 9). Similarly, plasma S100B was more than doubled in APP/PS1 mice.

The breakdown of the BBB and increased influx of TRL–Aβ in SFA-fed mice did not appear to be a consequence of exaggerated exposure to circulating Aβ, on the basis that the plasma concentration of $A\beta_{1-40}$ and $A\beta_{1-42}$ were similar for all groups of wild-type mice (Fig. 10).

Discussion

The present study suggests that chronic ingestion of SFA compromises BBB integrity, resulting in blood-to-brain delivery of plasma proteins, including apo B lipoproteins that may be endogenously enriched in Aβ. Consistent with the notion of BBB dysfunction in SFA-fed mice, there was evidence of cerebrospinal fluid-to-plasma efflux, with a doubling in the plasma concentration of S100B. The detrimental effect of SFA on BBB function seemed to be specific, as no differences were observed between control animals and mice supplemented with either MUFA or PUFA. The cerebral distribution and co-localisation of Aβ with apo B lipoproteins in SFA-fed mice was also found to be remarkably similar to that in APP/PS1 amyloid transgenic mice and in the latter there was a positive association of plasma-derived apo B lipoproteins with cerebral Aβ deposits. The cerebral distribution of apo B and IgG was greater at both 3 and 6 months of SFA feeding within the CTX than the HPF or BS regions, suggesting a site-specific effect. The observation is consistent with the hypothesis of enhanced blood-to-brain delivery of peripheral lipoprotein–Aβ, because the CTX has a more substantial capillary network. Kawai *et al.* reported that 60–77 % of amyloid plaques were associated with capillaries and relevant to the hypothesis presented in the present study, there was significantly greater vessel density within a 10 μm border surrounding plaques compared with unaffected grey matter[34]. Collectively, the findings of the present study provide one explanation of how dietary fats may influence AD risk. Ingestion of SFA could enhance peripheral delivery to the brain of circulating lipoprotein–Aβ and exacerbate the amyloidogenic cascade.

The fatty acid-enriched diets provided to mice were well tolerated and there were no significant differences in weight gain, plasma cholesterol or plasma TAG between groups. Therefore changes to BBB integrity in SFA-fed mice were not a consequence of dietary induced dyslipidaemia. The diets were physiologically relevant. Palmitic (16 : 0) and stearic (18 : 0) acid content of the mouse diets of 5·2 and 7·1 % of energy intake, respectively, is comparable with Western patterns of consumption of between 5 and 7 % for each. Total saturates of the mouse chow were also similar to Western dietary patterns (13 % mouse SFA chow *v.* 14 % in human diets) as well as total digestible energy as lipids (40 % for fatty acid-supplemented chow *v.* 37 % in Western diets).

We put forward the hypothesis that an SFA-induced elevation in plasma Aβ might compromise BBB function on

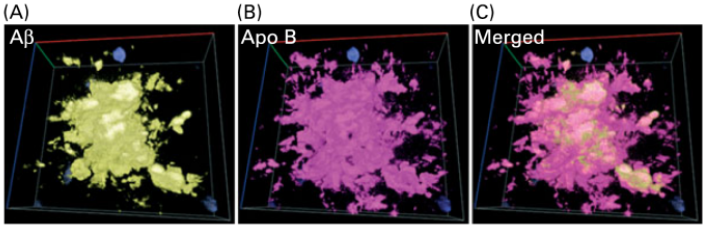


Fig. 6. Co-localisation of apo B with cerebral amyloid plaques. An immunofluorescent double-labelling method was utilised to explore the co-localisation of apo B with amyloid plaques in amyloid precursor protein/presenilin-1 (APP/PS1) transgenic Alzheimer's disease model mice. The distribution of apo B and amyloid-β (Aβ) for the same tissue specimen are shown separately (A and B). The co-location of apo B with Aβ is indicated in the merged image (C). For the latter, Aβ is shown in yellow and apo B is shown in magenta. Scales of *x* (red), *y* (blue) and *z* (green) axes are 70 × 70 × 22 μm.

BJN British Journal of Nutrition

NS British Journal of Nutrition

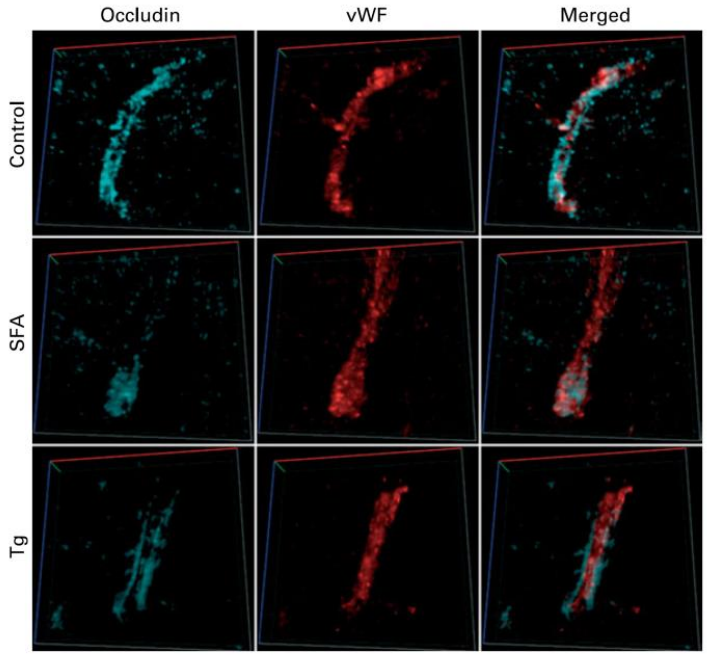


Fig. 7. Blood–brain barrier (BBB) three-dimensional (3-D) detection of the tight junction protein occludin. The expression of occludin-1 relative to epithelial cell abundance (expressed as von-Willebrand factor (vWF)) was quantitatively determined using double-labelling immunofluorescent microscopy. Representative 3-D images of occludin and vWF images are shown (x, y, $z = 80 \times 80 \times 7\ \mu m$). Significantly decreased expression of BBB occludin was found in SFA-fed mice compared with control mice, consistent with amyloid precursor protein/presenilin-1 (APP/PS1) amyloid trangenic (Tg) mice.

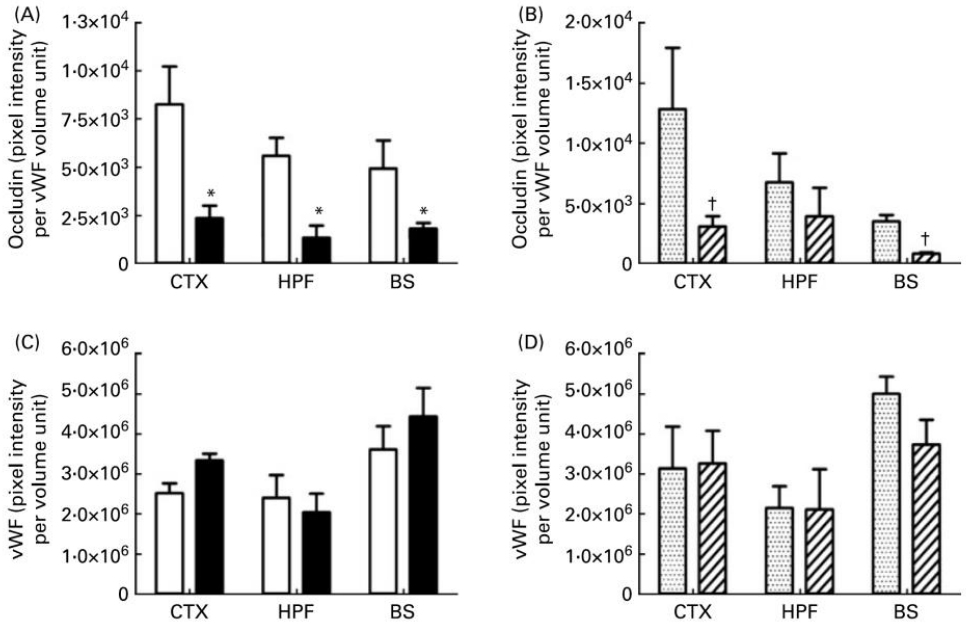


Fig. 8. Three-dimensional semi-quantitative analysis of cerebrovascular occludin (A and B) and von-Willebrand factor (vWF) (C and D) in control (□), SFA-fed (■), wild-type (▨) and amyloid precursor protein/presenilin-1 (APP/PS1) amyloid trangenic (▨) mice. Optical pixel intensities were measured in three major brain regions of the cortex (CTX), hippocampal formation (HPF) and brain stem (BS). Immunoreactivities of blood–brain barrier occludin-1 are expressed as per vWF volume unit. The net abundance of vWF is indicated per unit tissue. Values are means, with standard errors represented by vertical bars. * Mean value was significantly different from that of the control mice ($P < 0{\cdot}05$; t test). † Mean value was significantly different from that of the wild-type mice ($P < 0{\cdot}05$; t test).

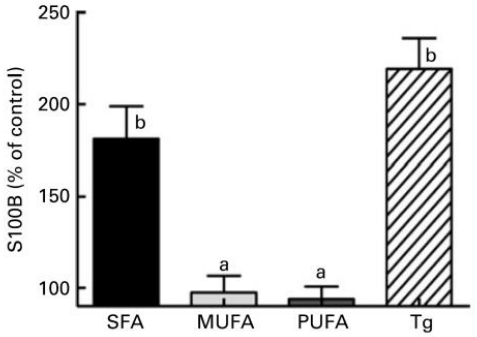


Fig. 9. Plasma level of S100B. Plasma S100B was determined by ELISA as a surrogate marker of brain-to-blood leakage. Mice fed SFA for 3 months had a significantly higher S100B level compared with the control, MUFA- and PUFA-fed mice. Similarly, amyloid precursor protein/presenilin-1 (APP/PS1) amyloid trangenic (Tg) mice had a doubling in plasma S100B compared with wild-type controls. Values are means, with standard errors represented by vertical bars. [a,b] Mean values with unlike letters were significantly different ($P<0{\cdot}01$; one-way ANOVA).

the basis that exogenous administration of this protein showed disturbing vasoreactive properties *in vivo*. However, in the present study the plasma concentrations of $A\beta_{1-40}$ and $A\beta_{1-42}$ were similar for all groups of wild-type mice. We cannot equivocally rule out an elevation in the lipoprotein pool of Aβ because some studies suggest that lipids block detection of lipoprotein-bound Aβ[35,36].

The SFA diet may have also compromised BBB function mechanisms independent of lipoprotein–Aβ concentration, including enhanced production of reactive oxygen species, increases in intracellular Ca or activation of endoplasmic reticulum stressors[37–40]. Clinical evidence suggests that inflammatory pathways can become activated because of impaired postprandial lipid metabolism[41]. Important differences in the cytotoxic effects of fatty acids have been reported, with longer-chain SFA being the most potent and the MUFA and PUFA being cytoprotective[37]. Morgan[37] suggests that the underlying toxicity of SFA is a consequence of disturbances in protein processing and endoplasmic reticulum dysfunction, for example, apoptotic induction. Consistent with this hypothesis, Patil *et al.* reported a palmitic acid-induced region-specific damage because of a higher fatty acid-metabolising capacity of cortical astroglia[42]. Conversely, cell-culture

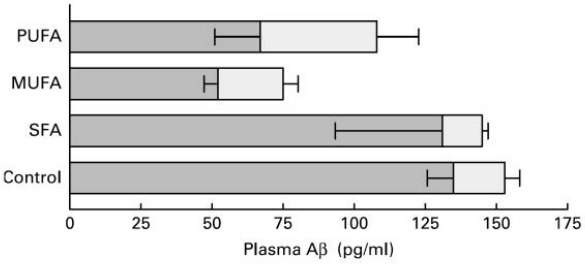


Fig. 10. Plasma amyloid-β (Aβ) concentration in control and high fatty acid-fed mice. Plasma levels of $A\beta_{1-40}$ (▩) and $A\beta_{1-42}$ (□) were measured with ELISA. Values are means, with standard errors represented by horizontal bars. There was no significant difference between the control group and any of the high-fat treatment groups.

studies suggest that incubation, particularly with longer-chain unsaturates, has an antagonistic effect on endoplasmic reticulum-centred stress pathways[43].

Dietary 'lipotoxicity' refers to the processes leading to end-organ damage and/or dysfunction following excess exposure to fatty acids and was first coined in the context of fat-induced insulin resistance[44]. Since then, however, the process has also been implicated in endothelial dysfunction and atherosclerosis, heart failure, kidney failure, steatohepatitis and liver failure, autoimmune inflammatory disorders, susceptibility to infections, cancer and ageing. The BBB disturbances identified in the present study may reflect a broader dietary toxic phenomenon.

SFA may also have compromised BBB function by secondary pathways other than elevations in plasma Aβ, or as a consequence of interactive effects with genes involved in Aβ metabolism and BBB function. For example, Deane *et al.* showed that apoE isoforms differentially regulate Aβ clearance from the brain by routing free Aβ through alternate receptors at the BBB. Apo E4-facilitated efflux was slower than apo E3- or apo E2-mediated clearance[45,46]. The effect of SFA on apo E isoforms was not explored in the present study.

Apo B immunoreactivity in senile plaque of subjects with AD was reported some years ago[47] but there was no evidence that this association was causal. However, several lines of study are consistent with the concept that peripheral delivery of lipoprotein–Aβ may contribute to AD risk[5,18,48–50]. Firstly, Aβ is tightly bound to TRL, the secretion of which is positively associated with the onset and progression of cerebrovascular and parenchymal amyloidosis[20–21]. Indirect evidence for the possibility of enhanced lipoprotein-mediated blood-to-brain delivery of Aβ is suggested by the study of Kreuter *et al.* who demonstrated enhanced drug transport into the brain when nanoparticles were covalently attached to apolipoproteins (including apo B)[51]. In clinical studies significantly greater levels of apo B are found in AD patients[52] and indirect evidence also comes from studies investigating the pro-atherogenic properties of apo B lipoproteins, a disease that shares some pathological similarities to AD. In arterial tissue, apo B lipoproteins and particularly the post-hydrolysed remnants of apo B lipoproteins have significant affinity for extracellular matrices and, if trapped, induce an inflammatory response[53]. The uptake by resident macrophages of apo B lipoproteins triggers a respiratory burst compromising cell viability leading to atherosclerotic plaque instability[54]. Obesity and diabetes also significantly increase risk for AD[55]. Profenno *et al.*[55] suggested that physiological changes common to obesity and diabetes plausibly promote AD. Resistance to the action of insulin in obese/diabetic individuals results in hepatic and intestinal apo B lipoprotein overproduction, diminished clearance of apo B lipoproteins from blood and postprandial dyslipidaemia[56]. It is possible that the cerebral parenchymal entrapment of lipoprotein–Aβ and formation of fibrillar deposits share pathways in common with the initiation of progression of atherosclerosis.

Nelson & Alkon[57] suggested that fibrillar formation of Aβ is prevented by proteins such as apo B that have affinity for the Aβ hydrophobic domain. Using a phage display system to explore protein–protein interaction, they found that Aβ binds to proteins primarily involved in LDL and cholesterol

transport. However, given that apo B is a protein not normally found in cerebrospinal fluid it is unlikely to serve as a suppressor of amyloidosis. Rather, cerebrovascular remodelling of apo B lipoprotein–Aβ delivered to the brain could exacerbate amyloidogenesis as a result of Aβ release, or enhanced epitope exposure[30].

There is an accumulating body of literature consistent with the concept that the onset and progression of AD is influenced by lifestyle factors including nutrition. Population studies support a role of dietary fats in AD. Laitinen *et al.* reported that intake of unsaturated fats is protective, whereas intake of saturates increases risk of AD[58]. In the Framingham study, the top quartile of plasma DHA was associated with a 47 % reduction in risk of all-cause dementia[59]. Furthermore, evidence continues to come from animal studies including by Oksman *et al.* who confirmed that saturates increase, while DHA decreases, cerebral Aβ levels compared with a soya oil diet[60].

The present study provides novel insight into how dietary fats might influence AD risk and reports for the first time differential effects of dietary fatty acids on BBB integrity. Mice chronically fed SFA show significant blood-to-brain delivery, retention and accumulation of apo B lipoproteins, primarily within the CTX of SFA-fed mice, observations consistent with the distribution of apo B in amyloid in brain specimens from subjects with AD. In the present study, chronic ingestion of SFA in wild-type mice also replicated the pattern of BBB dysfunction and of TRL–Aβ distribution observed in an established model of AD (APP/PS1 transgenics) maintained on normal chow. How dietary behaviour influences BBB function and the propensity for amyloidosis may prove helpful in the context of AD prevention.

Acknowledgements

The present study was financially supported by the National Health and Medical Research Council of Australia, and the Australian Technology Network Centre for Metabolic Fitness.

R. T. conducted the experimental procedures and prepared the manuscript. S. G. contributed to the animal care and experimental data collection. M. M. S. P.-G. contributed to experimental data collection. C. L. W. contributed to animal sample preparation. R. D. J. contributed to the development of methods. S. S. D. contributed to the experimental design and statistical assessment. J. C. L. M. contributed to the project concept, research funding, experimental design, data collection and writing of the manuscript.

The authors declare no conflicts of interest.

References

1. Pasinetti GM & Eberstein JA (2008) Metabolic syndrome and the role of dietary lifestyles in Alzheimer's disease. *J Neurochem* **106**, 1503–1514.
2. Kalmijn S (2000) Fatty acid intake and the risk of dementia and cognitive decline: a review of clinical and epidemiological studies. *J Nutr Health Aging* **4**, 202–207.
3. Petot GJ & Friedland RP (2004) Lipids, diet and Alzheimer's disease: an extended summary. *J Neurol Sci* **226**, 31–33.
4. Sparks DL, Scheff SW, Hunsaker JC III, *et al.* (1994) Induction of Alzheimer-like β-amyloid immunoreactivity in the brains of rabbits with dietary cholesterol. *Exp Neurol* **126**, 88–94.
5. Refolo LM, Malester B, LaFrancois J, *et al.* (2000) Hypercholesterolemia accelerates the Alzheimer's amyloid pathology in a transgenic mouse model. *Neurobiol Dis* **7**, 321–331.
6. Ellis RJ, Olichney JM, Thal LJ, *et al.* (1996) Cerebral amyloid angiopathy in the brains of patients with Alzheimer's disease: the CERAD experience, Part XV. *Neurology* **46**, 1592–1596.
7. Kalaria RN (1992) The blood–brain barrier and cerebral microcirculation in Alzheimer disease. *Cerebrovasc Brain Metab Rev* **4**, 226–260.
8. Wisniewski HM, Vorbrodt AW & Wegiel J (1997) Amyloid angiopathy and blood–brain barrier changes in Alzheimer's disease. *Ann NY Acad Sci* **826**, 161–172.
9. Cullern KM (1997) Perivascular astrocytes within Alzheimer's disease plaques. *Neuroreport* **8**, 1961–1966.
10. Itagaki S, McGeer PL, Akiyama H, *et al.* (1989) Relationship of microglia and astrocytes to amyloid deposits of Alzheimer disease. *J Neuroimmunol* **24**, 173–182.
11. Hachinski V & Munoz DG (1997) Cerebrovascular pathology in Alzheimer's disease: cause, effect or epiphenomenon? *Ann N Y Acad Sci* **826**, 1–6.
12. Joachim CL, Duffy LK, Morris JH, *et al.* (1988) Protein chemical and immunocytochemical studies of meningovascular β-amyloid protein in Alzheimer's disease and normal aging. *Brain Res* **474**, 100–111.
13. Deane R, Sagare A, Hamm K, *et al.* (2005) IgG-assisted age-dependent clearance of Alzheimer's amyloid β peptide by the blood–brain barrier neonatal Fc receptor. *J Neurosci* **25**, 11495–11503.
14. Mackic JB, Bading J, Ghiso J, *et al.* (2002) Circulating amyloid-β peptide crosses the blood–brain barrier in aged monkeys and contributes to Alzheimer's disease lesions. *Vascul Pharmacol* **38**, 303–313.
15. Crossgrove JS, Li GJ & Zheng W (2005) The choroid plexus removes β-amyloid from brain cerebrospinal fluid. *Exp Biol Med* **230**, 771–776.
16. Donahue JE, Flaherty SL, Johanson CE, *et al.* (2006) RAGE, LRP-1, and amyloid-β protein in Alzheimer's disease. *Acta Neuropathol* **112**, 405–415.
17. Galloway S, Jian L, Johnsen R, *et al.* (2007) β-Amyloid or its precursor protein is found in epithelial cells of the small intestine and is stimulated by high-fat feeding. *J Nutr Biochem* **18**, 279–284.
18. Takechi R, Galloway S, Pallebage-Gamarallage MMS, *et al.* (2008) Chylomicron amyloid-β in the aetiology of Alzheimer's disease. *Atheroscler Supp* **9**, 19–25.
19. Koudinov AR & Koudinova NV (1997) Alzheimer's soluble amyloid β protein is secreted by HepG2 cells as an apolipoprotein. *Cell Biol Int* **21**, 265–271.
20. James AP, Pal S, Gennat HC, *et al.* (2003) The incorporation and metabolism of amyloid-β into chylomicron-like lipid emulsions. *J Alzeimers Dis* **5**, 179–188.
21. Mamo JC, Jian L, James AP, *et al.* (2008) Plasma lipoprotein β-amyloid in subjects with Alzheimer's disease or mild cognitive impairment. *Ann Clin Biochem* **45**, 395–403.
22. Smith D, Watts GF, Dane-Stewart C, *et al.* (1999) Post-prandial chylomicron response can be predicted by a single measurement of plasma apolipoprotein B48 in the fasting state. *Eur J Clin Invest* **29**, 204–209.
23. Boyt AA, Taddei TK, Hallmayer J, *et al.* (2000) The effect of insulin and glucose on the plasma concentration of Alzheimer's amyloid precursor protein. *Neuroscience* **95**, 727–734.
24. LaRue B, Hogg E, Sagare A, *et al.* (2004) Method for measurement of the blood–brain barrier permeability in the perfused mouse brain: application to amyloid-β peptide in wild type

and Alzheimer's Tg2576 mice. *J Neurosci Methods* **138**, 233–242.
25. Burgess BL, McIsaac SA, Naus KE, *et al.* (2006) Elevated plasma triglyceride levels precede amyloid deposition in Alzheimer's disease mouse models with abundant A β in plasma. *Neurobiol Dis* **24**, 114–127.
26. Arendash GW, Su GC, Crawford FC, *et al.* (1999) Intravascular β-amyloid infusion increases blood pressure: implications for a vasoactive role of β-amyloid in the pathogenesis of Alzheimer's disease. *Neurosci Lett* **268**, 17–20.
27. Maness LM, Banks WA, Podlisny MB, *et al.* (1994) Passage of human amyloid β-protein $_{1-40}$ across the murine blood–brain barrier. *Life Sci* **55**, 1643–1650.
28. Thomas T, McLendon C, Sutton ET, *et al.* (1997) Cerebrovascualr endothelial dysfunction mediated by β-amyloid. *Neuroreport* **8**, 1387–1391.
29. Natah SS, Mouihate A, Pittman QJ, *et al.* (2005) Disruption of the blood–brain barrier during TNBS colitis. *Neurogastroenterol Motil* **17**, 433–446.
30. Kay AD, Day SP, Nicoll JA, *et al.* (2003) Remodelling of cerebrospinal fluid lipoproteins after subarachnoid hemorrhage. *Atherosclerosis* **170**, 141–146.
31. Takechi R, Galloway S, Pallebage-Gamarallage MM, *et al.* (2008) Three-dimensional immunofluorescent double labelling using polyclonal antibodies derived from the same species: enterocytic colocalization of chylomicrons with Golgi apparatus. *Histochem Cell Biol* **129**, 779–784.
32. Kleindienst A, Hesse F, Bullock MR, *et al.* (2007) The neurotrophic protein S100B: value as a marker of brain damage and possible therapeutic implications. *Prog Brain Res* **161**, 317–325.
33. Yang Q, Hou X, Hamberger A, *et al.* (1995) S-100 β immunoreactivity in neurons of the rat peripheral sensory ganglia. *Neuroreport* **6**, 2005–2009.
34. Kawai M, Kalaria RN, Harik SI, *et al.* (1990) The relationship of amyloid plaques to cerebral capillaries in Alzheimer's disease. *Am J Pathol* **137**, 1435–1446.
35. Yanagisawa K, McLaurin J, Michikawa M, *et al.* (1997) Amyloid β-protein (A β) associated with lipid molecules: immunoreactivity distinct from that of soluble A β. *FEBS Lett* **420**, 43–46.
36. James AP & Mamo JC (2005) The immunodetection of lipoprotein-bound amyloid-β is attenuated because of the presence of lipids. *Ann Clin Biochem* **42**, 70–72.
37. Morgan NG (2009) Fatty acids and β-cell toxicity. *Curr Opin Clin Nutr Metab Care* **12**, 117–122.
38. Carpentier AC (2008) Postprandial fatty acid metabolism in the development of lipotoxicity and type 2 diabetes. *Diabetes Metab* **34**, 97–107.
39. Cnop M, Igoillo-Esteve M, Chunha DA, *et al.* (2008) An update on lipotoxic endoplasmic reticulum stress in pancreatic β-cells. *Biochem Soc Trans* **36**, 909–915.
40. Solfrizzi V, Frisardi V, Capurso C, *et al.* (2009) Dietary fatty acids in dementia and predementia syndromes: epidemiological evidence and possible underlying mechanisms. *Ageing Res Rev* (epublication ahead of print version 28 July 2009).
41. Van oostrom AJ, Alipour A, Plokker TW, *et al.* (2007) The metabolic syndrome in relation to complement component 3 and postprandial lipemia in patients from an outpatient lipid clinic and healthy volunteers. *Atherosclerosis* **190**, 167–173.
42. Patil S, Balu D, Melrose J, *et al.* (2008) Brain region-specificity of palmitic acid-induced abnormalities associated with Alzheimer's disease. *BMC Res Notes* **1**, 20.
43. Diakogiannaki E & Morgan NG (2008) Differential regulation of the ER stress response by long-chain fatty acids in the pancreatic β-cell. *Biochem Soc Trans* **36**, 959–962.
44. Unger RH (1995) Lipotoxicity in the pathogenesis of obesity-dependent NIDDM. Genetic and clinical implications. *Diabetes* **44**, 863–870.
45. Deane R, Sagare A, Hamm K, *et al.* (2008) ApoE isoform-specific disruption of amyloid β peptide clearance from mouse brain. *J Clin Invest* **118**, 4002–4013.
46. Deane R, Bell RD, Sagare A, *et al.* (2009) Clearance of amyloid-β peptide across the blood–brain barrier: implication for therapies in Alzheimer's disease. *CNS Neurol Disord Drug Targets* **8**, 16–30.
47. Namba Y, Tsuchiya H & Ikeda K (1992) Apolipoprotein B immunoreactivity in senile plaque and vascular amyloids and neurofibrillary tangles in the brains of patients with Alzheimer's disease. *Neurosci Lett* **134**, 264–266.
48. Cole GM & Ard MD (2000) Influence of lipoproteins on microglial degradation of Alzheimer's amyloid β-protein. *Microsc Res Tech* **50**, 316–324.
49. Clifford PM, Zarrabi S, Siu G, *et al.* (2007) Aβ peptides can enter the brain through a defective blood–brain barrier and bind selectively to neurons. *Brain Res* **1142**, 223–236.
50. Takechi R, Galloway S, Pallebage-Gamarallage M, *et al.* (2009) Three-dimensional colocalization analysis of plasma-derived apolipoprotein B with amyloid plaques in APP/PS1 transgenic mice. *Histochem Cell Biol* **131**, 661–666.
51. Kreuter J, Hekmatara T, Dreis S, *et al.* (2007) Covalent attachment of apolipoprotein A-I and apolipoprotein B-100 to albumin nanoparticles enables drug transport into the brain. *J Control Release* **118**, 54–58.
52. Caramelli P, Nitrini R, Maranhao R, *et al.* (1999) Increased apolipoprotein B serum concentration in Alzheimer's disease. *Acta Neurol Scand* **100**, 61–63.
53. Mamo JC, Proctor SD & Smith D (1998) Retention of chylomicron remnants by arterial tissue; importance of an efficient clearance mechanism from plasma. *Atherosclerosis* **141**, S63–S69.
54. Elsegood CL, Pal S, Roach PD, *et al.* (2001) Binding and uptake of chylomicron remnants by primary and THP-1 human monocyte-derived macrophages: determination of binding proteins. *Clin Sci* **101**, 111–119.
55. Profenno LA, Porsteinsson AP & Faraone SV (2009) Meta-analysis of Alzheimer's disease risk with obesity, diabetes and related disorders. *Biol Psychiatry* (epublication ahead of print version 7 April 2009).
56. Mamo JC, Watts GF, Barrett PH, *et al.* (2001) Postprandial dyslipidemia in men with visceral obesity: an effect of reduced LDL receptor expression? *Am J Physiol Endocrinol Metab* **281**, E626–E632.
57. Nelson TJ & Alkon DL (2007) Protection against β-amyloid-induced apoptosis by peptides interacting with β-amyloid. *J Biol Chem* **282**, 31238–31249.
58. Laitinen MH, Ngandu T, Rovio S, *et al.* (2006) Fat intake at midlife and risk of dementia and Alzheimer's disease: a population based study. *Dement Geriatr Cogn Disord* **22**, 99–107.
59. Schaefer EJ, Bongard V, Beiser AS, *et al.* (2006) Plasma phosphatidylcholine docosahexaenoic acid content and risk of dementia and Alzheimer disease: the Framingham Heart Study. *Arch Neurol* **63**, 1545–1550.
60. Oksman M, Iivonen H, Hogyes E, *et al.* (2006) Impact of different saturated fatty acid, polyunsaturated fatty acid and cholesterol containing diets on β-amyloid accumulation in APP/PS1 trangenic mice. *Neurobiol Dis* **23**, 563–572.

NS British Journal of Nutrition

Article 5:

Takechi R, **Galloway S**, Pallebage-Gamarallage MM, Lam V, Mamo JCL. (2009) Dietary fats, cerebrovascular integrity and Alzheimer's disease risk. *Prog Lipid Res*. 49, 159-170 (Review)

Synopsis:

A collection of epidemiological, animal and cell studies has led to the notion that AD pathology is strongly linked to the metabolism of dietary fats. Although the exact mechanisms remain unclear it is thought that dietary fat plays a role in the modulation of Aβ, the main protein implicated in AD. This candidacy examines in detail the effects of dietary fat upon regulation of intestinal Aβ-apo B-lipoproteins. In the context of my candidacy, this review provides references for downstream pathological events and specifically explores pleiotropic effects of dietary fat and cholesterol on the various physiological and pathological mechanisms shown to exacerbate the onset and progression of AD. I have contributed intellectually to the content of this article as well as appraising the manuscript.

Section 1 (introduction) introduces the role of Aβ in AD pathology and describes the possible mechanisms behind elevated cerebral Aβ including over production of local Aβ, increased transport of peripheral Aβ into the brain and/or decreased Aβ clearance/degradation. Of these possible mechanisms, the author describes decreased Aβ clearance and increased Aβ production is less likely to be the case in sporadic AD cases. Increased BBB permeability and increased extravasation of plasma proteins within AD brains has been reported (Ellis et al. 1996, Wisniewski et al. 1997a) and this article suggests that dietary fat is possibly implicated. Saturated fat and cholesterol have both been previously implicated with greater risk of developing AD (Oba et al. 2009, Yusuf et al. 2004, Menotti et al. 1999, Singh et al. 2002, Vincent-Baudry et al. 2005, De Lorgeril et al. 1996, de Lorgeril et al. 1999) and animal models show aggressive cerebral pathology in response to increased SFA and cholesterol feeding (Sparks et al. 1994, Oksman et al. 2006, Refolo et al. 2000, Shie et al. 2002).

Section 2 provides a summary of epidemiological studies into the effects of dietary fatty acids (SFA, MUFA and n3/n6 PUFA) on cognitive decline and AD (See Chapter 1 Literature Review) as well as the effects of SFA diet on enterocyte derived Aβ. In brief, epidemiological studies have found intake of SFA to increase risk and unsaturated fat to decrease risk of cognitive decline and developing AD (Laitinen et al. 2006). The Mediterranean diet which is low in SFA and cholesterol and high in MUFA (predominantly through olive oil) has been shown to be beneficial towards chronic disease, cognitive decline and AD (Oba et al. 2009, Vincent-Baudry et al. 2005, Scarmeas et al. 2009, Petot et al. 2004). Polyunsaturated fats have been of particular interest in relation to mental health as about 50% of brain fatty acids are composed of n3s, DHA and EPA. Supplementation of diet with fish oil high in n-3 DHA has been shown to be beneficial in reducing cognitive decline (Kalmijn 2000, Scarmeas et al. 2009, Cunnane et al. 2009).

Consistent with previous epidemiological studies laboratory research shows that Aβ, the primary protein implicated in AD associates with lipoproteins in liver cells (Koudinov and Koudinova 1997) and in plasma, binds primarily to lipoproteins enriched with triglycerides (Mamo et al. 2008). The small intestine has also been shown to contribute to the plasma pool of Aβ and small intestinal enterocytes can express Aβ within the perinuclear location of the CM assembly. In addition, intracellular Aβ expression has been found to co-localize with apo B, the obligatory component of primordial CM molecules (Galloway et al. 2007, Galloway et al. 2008, Pallebage-Gamarallage et al. 2009). Production, secretion and transport of Aβ parallel those of a CM molecule and Aβ may be physiologically involved in metabolism of these lipoproteins. Accumulation of CMs after a high-fat meal (post prandial) has been shown to be increased in subjects with AD and cognitive decline (Boyt et al. 1999). Increased abundance of enterocytic Aβ induced by SFA and cholesterol feeding can translate into accumulation of Aβ-lipoproteins as a result of suppressive effects of SFA on the clearance of apo B-containing lipoproteins via reduced receptor expression (Jackson et al. 2006, Roberts et al. 2002, Hayes et al. 1997). Collectively these studies suggest that a diet high in SFA may induce an increase in enterocytic Aβ production and secretion into blood and a lowered postprandial Aβ clearance rate leading to increased plasma Aβ-apo B-lipoproteins (TRL-Aβ). Increased plasma levels of Aβ-apo B-lipoproteins can translate into concentration dependent transport into the brain and present an early pathological

pathway of AD progression. The presence of apo B within amyloid plaques of human brain specimens (Namba et al. 1992) strongly supports this hypothesis.

Section 3 explores the currently known mechanisms for Aβ transport across the BBB and examines the effects of SFA and cholesterol feeding on BBB integrity. Receptor mediated mechanisms for Aβ-TRL are currently unclear but may include transport via advanced glycosylation end products (RAGE) (Deane et al. 2003, Donhaue et al. 2006) and low-density-receptor related protein-1 (LRP1) (Deane et al. 2005, Deane et al. 2009, Donahue et al. 2006). In addition, the balance of RAGE relative to LRP1 may be important in regard to cerebral homeostasis as RAGE determines influx and LRP1 is more implicated in efflux of Aβ from the brain (Deane et al. 2009, Donahue et al. 2006, Deane et al. 2008, Ye et al. 2005).

Indeed, the transport of plasma Aβ-TRL across the brain could also be severely affected if the BBB is compromised, allowing increased influx due to aberrations in endothelial cell and astrocyte functions Aβ has shown to have "vasoactive" effects on the BBB including damage to endothelial cells (Thomas et al. 1997) and glial cells (George et al. 2004) resulting in a more permeable BBB. Chronic increases in plasma Aβ-TRL induced by high dietary SFA intake could therefore notionally damage cerebral blood vessels. One of the author's own publications (described in article 4), shows that SFA feeding, compared to MUFA, PUFA and low-fat control diets showed marked leakage of the BBB, resulting in an increase in bi-directional transport of proteins across the BBB. Saturated fat feeding specifically decreased expression of junction protein occludin along with increases in influx of plasma apo B/IgG and increased efflux of S100β (Takechi et al. 2009). Although specific blood to brain influx of Aβ-TRL was not explored, cerebrovascular presence of apo B co-localised with Aβ indicates a high likelihood of Aβ-TRL transport.

Section 4 explores other mechanisms by which SFA and cholesterol can contribute to BBB damage. As plasma Aβ results were not conclusive, the significance of SFA induced increased Aβ-TRL towards BBB integrity remains unclear at this point. In addition, SFA could damage BBB via other mechanisms. Increased exposure of BBB to fatty acids may induce greater levels of "lipotoxicity" causing endothelial dysfunction such that long chain SFAs are toxic compared to

MUFA and PUFA (Morgan 2009). Saturated fatty acids causes cell damage by altering endoplasmic reticulum processes including stress pathways (Diakogiannaki and Morgan 2008) and apoptosis (Morgan 2009). In addition, dietary fat induced lipid and protein oxidation has been reported (Studzinski et al. 2009, Ronti et al. 2006). Membrane lipid composition has also been reported to be influenced by dietary fats via alteration of phospholipid levels and lipid rafts of neurons (Wassall and Stillwell 2009, Wassall et al. 2004, Diaz et al. 2002). Dietary fat may also influence changes in genetic expression of key Aβ regulatory proteins (Puskas et al. 2003). In addition, SFA can dilate blood vessels and increased blood flow to the brain could foresee increased influx of Aβ into the brain. The effect of exogenous (dietary) cholesterol on BBB integrity was also reported (Ghribi et al. 2006). Research from our laboratory confirms that similar to SFA feeding, cholesterol feeding resulted in an increase in extravasation of apo B from blood to brain (Takechi et al. 2010b). Like SFA, intracellular cholesterol in higher than physiological concentrations, can also cause apoptosis by inducing ER and mitochondrial stress (Feng et al. 2003, Yao and Tabas 2001). However, cholesterol can also cause damage through different mechanisms to SFA including by reduction of Aβ production (Abad-Rodriguez et al. 2004, Pallebage-Gamarallage et al. 2009), increased secretion (Frears et al. 1999), increased binding to cell membrane and accelerated oligomerization (Subasinghe et al. 2003).

In section 5, the author discusses one of the key proteins which influences risk of developing AD, apo E4, and discusses the implications of apo E isoforms on TRL metabolism and related toxic mechanisms. Compared to other isoforms, apo E4 increases risk of developing AD by 17 % or 43 % dependant on whether an individual contains one or two alleles (Strittmatter and Roses 1996). The involvement of Apo E4 in relation to AD has been reported (Boyt et al. 1999, Donahue and Johanson 2008, Fryer et al. 2005, Irizarry et al. 2004, LaDu et al. 1997, Mahley and Huang 1999, Poirier et al. 1993, Strittmatter et al. 1993a, Refolo and Fillit 2004, Jofre-Monseny et al. 2008) and specifically in relation to Aβ metabolism, inflammation and oxidation, cholesterol homeostasis (see above), maintenance of BBB integrity and neuron function. Apolipoprotein E4 could be implicated in AD pathology via by facilitating the blood to brain transport and parenchyma deposition of Aβ-TRL. Apo E4 patients report “leaky” BBB compared to E2 and E3 subjects and under such circumstances this can lead to an increase in

the influx of plasma Aβ-TRL (Deane et al. 2008). Physiologically, apo E influences the metabolism of TRL by binding to remnants (Mahley 1988, Hatters et al. 2006) and LDL receptor (Heeren et al. 2002, Krapp et al. 1996, Mamo et al. 1991). Compared to apo E3 and apo E2 which associates primarily with hepatically derived lipoproteins, apo E4 preferentially binds to TRL namely CMs (Saito et al. 2003) and comprises up to 65 % (total mass) of lipoproteins (Campos et al. 1992). The presence of apo E on Aβ-TRL can facilitate nucleation into brain matrices as apo E binds with high affinity to HSPG. In addition, apo E is an important ligand for the binding of TRL remnants to LRP1 which is implicated in cerebral Aβ efflux. However, cell culture studies indicate that the binding affinity of apo E isoforms to LRP is not different, indicating that efflux is relatively constant and efflux mechanisms may not be implicated in AD via this clearance route.

Section 6 concludes important questions as to how cerebral Aβ-TRL can deposit into the brain by exploring the possible association between apo B and various proteoglycans found in extracellular matrices of the brain. Proteoglycans, the predominant component of extracellular matrices can bind to both apo B and apo E (Flood et al. 2002, Bame et al. 1997). Studies have found HSPG, a type of proteoglycan, to be involved in the formation of amyloid plaques (van Horssen et al. 2003). Agrin, perlecan, biglycan and decorin are all proteoglycans which are capable of binding to apo B/apo E and these have been implicated in retention of lipoprotein (Iozzo 1998, Small et al. 1996, O'Brien et al. 1998, Olin et al. 2001). Recently, using immune-based approach, Lam V et al has recently studied the colocalization of Aβ-apo B with agrin, perlecan, biglycan and decorin in a transgenic (APP/PS1) mouse model of AD and found perlecan, biglycan and decorin to be co-localized with apo B and Aβ in amyloid plaques (Fig 3) (Lam unpublished data). [Note the "unpublished data" was published in Neuroscience letters (Lam et al. 2011)].

Section 6 shows that apo B derived from the small intestine and/or liver is present in the brain and amyloid plaques, which leads to the question as to whether small intestine or liver derived Aβ-apo B contributes primarily to BBB damage.

Section 7 investigates the role of the small intestine and liver on apo B-lipoprotein and BBB integrity on AD risk. In mice, both the liver and the small

intestine produce apo B48 whereas in humans apo B48 is exclusively present on CMs (Isherwood et al. 1997, van Greevenbroek and de Bruin 1998) and also exclusively produced by enterocytes; apo B100 is produced mainly in hepatocytes. Apolipoprotein B48 is 48 % of the amino acid sequence of apo B100 and may influence differences in CM metabolism compared to apo B100-containing lipoproteins. Production and secretion of VLDL from hepatocytes is a constant process, whereas production and secretion of CMs from enterocytes is largely dependent on the availability of triglycerides and cholesterol from dietary sources. In clinical studies of AD subjects, post-prandial (or CM) clearance was reduced compared to controls, whereas fasting levels of apo B were not significantly changed (Mamo et al. 2008, Caramelli et al. 1999). The post absorptive state primarily indicates an increase in secretion of apo B48-CMs (small intestinal origin) whereas fasting apo B levels reflect liver-derived lipoproteins. Our studies have found that enterocytic Aβ is highly responsive to the availability of substrate from diet; fasting led to abolishment of expression, LF diet led to consistent Aβ expression and HF feeding led to significant increases in perinuclear Aβ expression. These observations are consistent with dietary fat influences on production of Aβ from APP with apo B. Saturated fat feeding induced increase in intestinal abundance of Aβ is indicative that intestinal apo B48 is more significantly influenced by diet than apo B48 of liver origin. However, this does not rule out hepatic contribution per se. The secretion of Aβ-lipoproteins from enterocytes represents the majority of the post-absorptive pool of plasma lipoproteins compared to greater apo B100/apo B48 when post-absorptive state is not reached. In addition, hydrolysis of apo B48-lipoproteins is carried out at a greater rate compared to hepatic-apo B100 lipoproteins; CM remnants are cleared in about half the time compared to VLDL remnant clearance.

The roles of apo B48 and apo B100 have been previously studied in atherosclerotic plaques. Although both apo B48 and apo B100 have been found to penetrate endothelial cells and reside in the tunica intima/media of vessels (Proctor et al. 2002, Olofsson and Boren 2005), apo B48 was substantially more invasive and was present in greater abundance (Pal et al. 2003). The increase in affinity of apo B48 to arterial wall was found to be specific and not dependent on plasma concentrations (Pal et al. 2003). Notionally the extent of BBB damage due to increased exposure should not be different between intestinally or hepatically

derived lipoproteins. Both apo B48 and apo B100 can bind HSPGs (Proctor and Mamo 2003), however it is not known if increased arterial retention of apo B48-lipoproteins are also extended to greater retention by extracellular matrices of the brain. Currently, evidence suggests that intestinally derived Aβ contributes to greater plasma levels of apo B-containing lipoproteins postprandially, however liver derived lipoproteins are present in greater concentrations in blood throughout the majority of the day and therefore, their pathological contribution towards AD cannot be excluded.

In summary, this review examines current research on AD and lipids and dietary fat metabolism and proposes a novel insight into the pathology of AD in relation to dietary fats. Dietary SFA can specifically increase risk of developing AD by increasing production and secretion of Aβ-lipoprotein. SFA induced damage to BBB integrity can lead to an increase in extravasation of plasma Aβ-apo B-lipoproteins which have high affinity to brain HSPGs. Understanding the mechanisms underlying dietary fatty acids (and cholesterol) involvement in AD pathology could be of importance when considering strategies for the future prevention and maintenance of AD and cognitive decline and therapeutic interventions in AD, whether pharmacological, nutritional or lifestyle based should espouse both lipid metabolism and cerebrovascular integrity as key considerations in disease prevention and/or maintenance.

Progress in Lipid Research 49 (2010) 159–170

Contents lists available at ScienceDirect

Progress in Lipid Research

journal homepage: www.elsevier.com/locate/plipres

Review

Dietary fats, cerebrovasculature integrity and Alzheimer's disease risk

R. Takechi, S. Galloway, M.M.S. Pallebage-Gamarallage, V. Lam, J.C.L. Mamo *

School of Public Health, Curtin Health Innovation Research Institute, the Australian Technology Network-Centre for Metabolic Fitness, Curtin University of Technology, Bentley Campus, Kent Street, Perth 6102, Australia

ARTICLE INFO

Article history:
Received 15 October 2009
Received in revised form 30 October 2009
Accepted 30 October 2009

Keywords:
Alzheimer's disease
Amyloid-β
Blood–brain barrier
Cerebrovasculature
Chylomicrons
Dietary-fats
Enterocytes
Inflammation
Proteoglycans
Saturated fatty acids
Triacylglycerol-rich-lipoproteins

ABSTRACT

An emerging body of evidence is consistent with the hypothesis that dietary fats influence Alzheimer's disease (AD) risk, but less clear is the mechanisms by which this occurs. Alzheimer's is an inflammatory disorder, many consider in response to fibrillar formation and extracellular deposition of amyloid-beta (Aβ). Alternatively, amyloidosis could notionally be a secondary phenomenon to inflammation, because some studies suggest that cerebrovascular disturbances precede amyloid plaque formation. Hence, dietary fats may influence AD risk by either modulating Aβ metabolism, or via Aβ independent pathways. This review explores these two possibilities taking into consideration; (i) the substantial affinity of Aβ for lipids and its ordinary metabolism as an apolipoprotein; (ii) evidence that Aβ has potent vasoactive properties and (iii) studies which show that dietary fats modulate Aβ biogenesis and secretion. We discuss accumulating evidence that dietary fats significantly influence cerebrovascular integrity and as a consequence altered Aβ kinetics across the blood–brain barrier (BBB). Specifically, chronic ingestion of saturated fats or cholesterol appears to results in BBB dysfunction and exaggerated delivery from blood-to-brain of peripheral Aβ associated with lipoproteins of intestinal and hepatic origin. Interestingly, the pattern of saturated fat/cholesterol induced cerebrovascular disturbances in otherwise normal wild-type animal strains is analogous to established models of AD genetically modified to overproduce Aβ, consistent with a causal association. Saturated fats and cholesterol may exacerbate Aβ induced cerebrovascular disturbances by enhancing exposure of vessels of circulating Aβ. However, presently there is no evidence to support this contention. Rather, SFA and cholesterol appear to more broadly compromise BBB integrity with the consequence of plasma protein leakage into brain, including lipoprotein associated Aβ. The latter findings are consistent with the concept that AD is a dietary-fat induced phenotype of vascular dementia, reflecting the extraordinary entrapment of peripherally derived lipoproteins endogenously enriched in Aβ. Rather than being the initiating trigger for inflammation in AD, accumulation of extracellular lipoprotein-Aβ may be a secondary amplifier of dietary induced inflammation, or possibly, simply be consequential. Clearly, delineating the mechanisms by which dietary fats increase AD risk may be informative in developing new strategies for prevention and treatment of AD.

Contents

Abbreviations: Aβ, amyloid-β; AD, Alzheimer's disease; apo, apolipoprotein; BBB, blood–brain barrier; CSF, cerebrospinal fluid; CVD, cardiovascular disease; DHA, docosahexanoic acid; EPA, eicosapentaenoic acid; GAG, glycosaminoglycans; HSPG, heparin sulphate proteoglycan; IgG, immunoglobulin G; LDL-r, low density lipoprotein receptor; LRP1, lipoprotein receptor related protein-1; MCI, mild cognitive impairmend; MUFA, monounsaturated fatty acid; PUFA, poly-unsaturated fatty acid; RAGE, receptor for advanced glycosylation end products; SFA, saturated fatty acid; TAG, triacylglycerol; TRL, TAG rich lipoprotein; VLDL, very low density lipoprotein.

* Corresponding author. Tel.: +61 8 92667232; fax: +61 8 92662958.
E-mail address: J.Mamo@Curtin.edu.au (J.C.L. Mamo).

0163-7827/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.plipres.2009.10.004

1. Introduction

Hallmark pathological characteristics of advanced Alzheimer's disease (AD) include hyperphosphorylation of the microtubular protein tau in neurons and extracellular deposits of protein that are enriched in the protein amyloid-beta (Aβ) [1,2]. The formation of tau-tangles results in neuronal synapse dysfunction and eventually loss of cell-cell communication, whereas disturbed Aβ kinetics may be pivotal to pro-inflammatory pathways that compromise cellular integrity [1,3,4]. Despite a substantive body of research, it is presently difficult to equivocally delineate if these pathological features of AD are causal or consequential [5,6], emphasising the therapeutic challenge of identifying the inflammatory triggers that compromise cellular integrity.

Earlier research primarily focussed on the neuronal biogenesis of Aβ in the context that overproduction may initiate formation of fibrillar Aβ deposits and thereafter inflammation [7–10]. All mutations known to cause AD increases the production of Aβ peptide. However, in sporadic and late onset AD, the most common form of AD, Aβ biosynthesis is comparable to otherwise healthy individuals [11]. Alternatively, insufficient removal of Aβ from cerebrospinal fluid (CSF) has also been proposed as a mechanism for Aβ oligomerization [12–14]. However, the brain seems potently equipped with substantive efflux processes that would otherwise prevent this. It is estimated that CSF is replenished some three times daily via the choroid plexus and indeed the epithelial cells of the choroid plexus host an array of enzymes which effectively hydrolyse potentially toxic proteins including Aβ [15]. In addition, the endothelial cells of the cerebrovasculature host receptor-proteins that permit reciprocal transfer of Aβ across the blood–brain barrier (BBB) [16–20]. Collectively, there seems to be exquisite cerebral Aβ homeostatic mechanisms and therefore the concept that cerebral Aβ-overload triggers inflammatory pathways seems physiologically unlikely.

Alzheimer's disease is a chronic disorder and shares risk factors with other diseases such as non-insulin dependent diabetes and cardiovascular disease (CVD) [21–25]. However, chronic diseases are often 'spectrum disorders' with multiple aetiology. For example, obesity is a major risk factor for diabetes and CVD [26,27], but not a requisite feature per se and 40% of subjects who experience a coronary event are normolipaemic [26,27]. Indeed, cholesterol infiltration is not always found in atherosclerotic plaque and there is substantial heterogeneity in the extent of smooth muscle cell proliferation and tissue calcification [28,29]. Such paradoxes raise the possibility that amyloidosis is simply one of many 'triggers' for dementia per se.

Common to chronic disorders, there is ample evidence that lifestyle influences AD risk and progression. Good nutrition, physical activity and environmental enrichment confer synergistic reduction in AD risk [30–37]. However, in a therapeutic context, less is known of the efficacy of lifestyle interventions on disease progression, perhaps confounded by the diversity of dementia phenotypes. Given that within 20 years the expected global health burden for dementia, of which AD accounts for 80%, will exceed treatment of any other chronic disease [38–40], exploring lifestyle therapies has become as much an economic imperative as a therapeutic priority.

Most AD research has focused on damage of neurons, however there is an increasing effort to understand the possibility of cerebrovascular dysfunction as a primary risk factor for AD. This paradigm shift is arguably warranted because vascular alterations including endothelial and smooth muscle cell proliferation precede frank amyloidosis [41]. Blood plasma proteins have been detected in the parenchyma of AD brains [42,43] and inflammatory sequelae are commonly reported [44,45], observations that are consistent with breakdown of the BBB. Targeting vascular disturbances rather than Aβ deposition may therefore be an appropriate first-focus strategy for prevention and treatment of AD.

It is reasonable to suggest that diet is important in maintaining cerebrovascular integrity [46] particularly given the overwhelming evidence that it contributes substantially to coronary artery health and CVD risk [47–53]. Population studies also generally support this contention. Saturated fats and cholesterol are both positively associated with AD risk [54–60] and in animal models, including amyloid transgenic mice, saturated fat (SFA) and cholesterol induce or exacerbate cerebral amyloidosis [61–64]. The studies in transgenic amyloid mice are certainly consistent with a vascular contribution to disease over and above exaggerated Aβ biogenesis.

The purpose of this review is to provide contemporary consideration of the mechanisms by which dietary fats influence AD risk. Specifically, this article will focus on the putative interrelationship between plasma lipoproteins, peripheral Aβ kinetics and cerebrovasculature integrity.

2. Dietary fats and Alzheimer's disease risk

2.1. Population, clinical and animal model studies

Population studies support a role of dietary fats in AD, although this remains controversial. Laitinen reported that intake of unsaturated fats is protective, whereas intake of saturates increases risk of AD [60]. In the Framingham study, the top quartile of plasma docosahexanoic acid (DHA) (profoundly influenced by diet) was

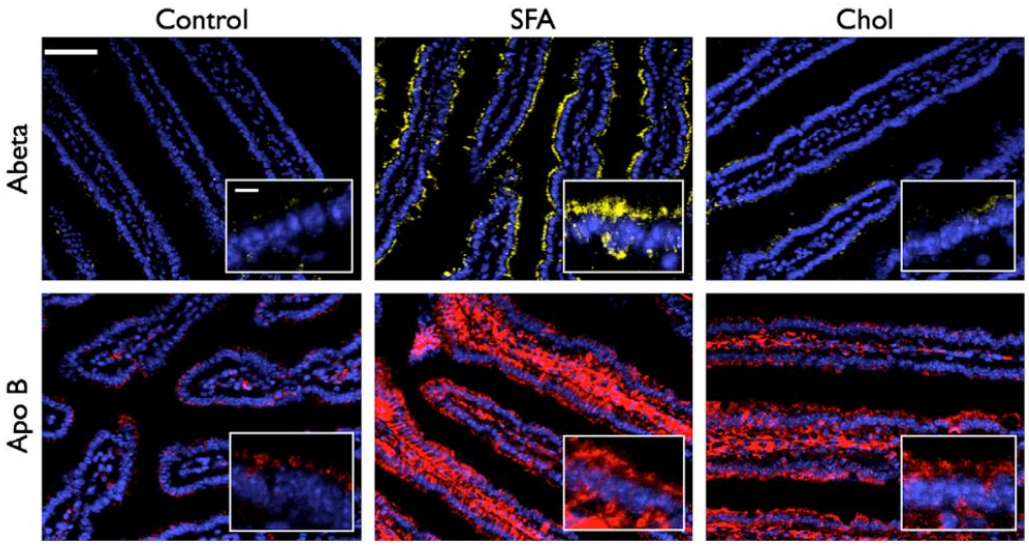


Fig. 1. Small intestine enterocytic abundance of amyloid-β and apolipoprotein B in saturated fat or cholesterol supplemented mice. The enterocytic perinuclear production and secretion into the lacteal of amyoid-β (Aβ) (top row) and apo B (bottom row) are shown in mice fed either a low-fat control, saturated fat enriched (SFA) or cholesterol supplemented (Chol) diet for 6 months. There was a significant increase of Aβ abundance concomitant with apo B in SFA-fed mice compared to control group. The cholesterol supplemented diet increased apo B abundance but had no significant effect on Aβ. Scale bar indicates 50 μm. Magnified images are also shown in each insert with 5 μm scale bar.

associated with a 47% reduction in risk of all-cause dementia [65]. Strong evidence continues to come from animal studies. Many studies show that cerebral amyloid burden and Alzheimer-like pathology is attenuated by a diet enriched in DHA in amyloid double transgenic [62,66–69]. Not surprisingly such findings have stimulated research to explore the benefits of diets low in SFA and cholesterol, such as the Mediterranean diet [47,51,70,71]. The omega 3 and 6 poly-unsaturated fatty acids (*n*3/*n*6 PUFA) have attracted particular interest. DHA and eicosapentaenoic acid (EPA) make up some 40–50% of cerebral fatty acids and are critical to neuronal plasticity and in addition may be therapeutically beneficial because of anti-inflammatory properties [69,72]. An increasing number of clinical studies demonstrate better cognitive performance in subjects with AD receiving *n*3 fatty acid supplementation [73] and meta-analysis is consistent with such a purported benefit [56,70]. However, the evidence that *n*3/6 intake reduces prevalence of AD is presently less convincing [74]. The mechanisms by which *n*3/6 confer protection have been elegantly summarized and will not be detailed here, suffice to say that these include the prevention of neuronal cell death, regulation of gene expression and anti-oxidative and anti-inflammatory functions [75–80].

2.2. Saturated fatty acids, amyloid-beta and the small intestine

The mechanisms by which dietary fats such as SFA increase AD risk may seem less of a scientific priority to delineate compared to dietary compounds that confer protection. Yet in some chronic disorders this approach has proven pivotal to developing effective therapeutic strategies for prevention and treatment of disease. For example, elucidating the role of cholesterol in atherosclerosis and cardiovascular disease led to the evolution of relatively safe and effective cholesterol-lowering drugs. Hence, significant attention will be provided in this article of possible pathways by which dietary fats increase AD risk and as a hypothesis-generating exercise.

Amyloid-beta is an amphiphylic protein normally chaperoned by transporter proteins [81]. However, strong hydrophobic domains have made distributional analysis of Aβ in blood and tissues difficult, because lipids often mask the immunodetection methodologies used to measure concentration [82,83]. Exogenous addition to blood of pre-solubilised Aβ suggests that less than 5% of Aβ binds to lipoproteins [84]. However, plasma lipoprotein fractionation and delipidation reveal that significant quantities of endogenous Aβ are associated with lipoproteins, particularly those enriched in triacylglycerol (TAG) [85]. The latter is also supported by studies in cell culture, which demonstrated that hepatocytes secrete Aβ as a lipoprotein complex [86].

Absorptive epithelial cells of the small intestine were more recently identified as another potential significant source of plasma Aβ [87–90] (Fig. 1). Enterocytic Aβ is enriched in the perinuclear region and within the lacteals, the site of chylomicron assembly and secretion respectively [90,91] and Aβ colocalizes with apolipoprotein (apo) B, an obligatory component of nascent chylomicrons (Fig. 1). Oral fat challenges in healthy subjects demonstrate a post-prandial Aβ response [92] and plasma kinetics of chylomicron-Aβ exactly parallels the metabolic pathway of the lipoprotein particle [93]. Indeed, given that Aβ significantly inhibits hepatic uptake of chylomicrons, one of its physiological roles is probably as a regulating apolipoprotein of TAG-rich lipoproteins (TRLs).

Dietary-fat regulation of enterocytic Aβ production and secretion was demonstrated in wild-type mice fed a SFA-enriched diet. Compared to low-fat fed controls, SFA substantially increased enterocytic Aβ, whereas fasting completely abolished Aβ immunoreactivity [87]. On the basis that SFA also suppress expression of receptors that are responsible for clearance of apo B lipoproteins [94–96], the findings raise the intriguing notion that dietary SFA may induce a state of post-prandial-hyperamyloidemia. Aberrations in chylomicron kinetics have been commonly reported in subjects with or at risk of CVD including otherwise normolipaemic subjects [97,98]. Chylomicron remnants are found in atherosclerotic plaque and may contribute significaintly to cholesterol deposition and inflammatory pathways [28,99,100]. Several lines of evidence are consistent with the notion that chylomicron-hyperamyloidemia may also contribute directly to amyloidosis and AD risk. Firstly, in clinical studies,

subjects with AD or mild cognitive impairment (MCI) have significantly greater plasma Aβ in a plasma TRL fraction that includes chylomicrons [85]. Moreover, plasma apo B_{48} (an exclusive marker of chylomicrons) was increased fourfold in post-absorptive AD subjects compared to age matched controls [85]. In amyloid transgenic mice that are established models of AD, onset and progression of disease was found to strongly correlate with secretion into blood of TRL-Aβ and plasma TRL-Aβ concentration [101]. In addition, studies showed apo B immunoreactivity associated with amyloid plaque of human brain specimens [102]. However the latter was not considered in the context that it may have reflected blood-to-brain delivery of plasma lipoprotein derived Aβ.

3. Blood-to-brain delivery of triacylglycerol-rich-lipoprotein amyloid-beta

3.1. Receptor mediated cerbrovascular amyloid-beta kinetics

The receptor for advanced glycosylation end products (RAGE) is one endothelial cell protein found to facilitate Aβ transfer from blood-to-brain [16,17]. However, there is no evidence that TRL or lipoproteins per se bind to RAGE, requiring therefore transfer of Aβ from the lipoprotein particle to the aqueous mileu prior to transport via this pathway. However, in vivo and in vitro studies suggest that Aβ binds tightly to TRL and is not shed or transferred to other chaperone transporters [93], so RAGE-mediated transfer of lipoprotein derived Aβ seems unlikely.

The low-density-lipoprotein receptor related protein-1 (LRP1) is expressed within the cerebrovascular endothelial cell junctions and binds with substantial affinity to both 'free' Aβ as well as to TRL-remnant lipoproteins that have become depleted of TAG. However, LRP1 is considered to be principally involved in cerebral efflux of Aβ to blood rather than influx of Aβ from plasma [12,13,17]. In AD subjects and animal models there appears to be a shift in RAGE relative to LRP expression [13,17,103,104], consistent with the possibility of a gradient shift of Aβ kinetics from blood-to-brain, but it is presently unclear if this pathway is relevant to TRL-Aβ metabolism.

3.2. Vasoactive active properties of amyloid-beta

Cerebral extravasation of TRL-Aβ may also occur non-specifically because of broader disruption of the cerebrovasculature. Plasma proteins, including apo B, have been reported in CSF and parenchyma of subjects with AD [42,44,102,105]. Indeed, raised TRL-Aβ may be responsible of the vascular disturbances which lead to increased rates of peripheral delivery [101,105,106]. Indirect evidence that TRL-Aβ may have vasoactive properties is provided by cell culture and animal model studies which investigated the effect of exogenous Aβ administration. Intravascular administration of solubilised Aβ induces significant endothelial cell damage with changes in the cell membrane, cytoplasm, nucleus and other organelles [107]. Sequestration of Aβ within brain capillaries was reported in several studies [18–20] and longer term administration of Aβ (2 weeks), resulted in a significantly compromised BBB and activated central-nervous-system glial cells [108].

The exposure of blood vessels to exogenous Aβ induces enhanced vasoconstriction and diminished vasodilation accompanied with lack of elasticity that are commonly seen in aged animals. In a study by Thomas et al. loss of endothelial function was demonstrated with acute Aβ exposure of bovine cerebral middle artery [107]. This vascular damage was prevented by the anti-oxidant enzyme superoxide-dismutase and the free radical scavenger PBN12, suggesting that reactive oxygen species may be involved in the vasoconstrictive action of Aβ. Morphological disturbances including necrotic cell damage accompanied the inflammatory response induced by Aβ exposure.

Animal model studies confirm blood-to-brain delivery of Aβ when the plasma concentration is chronically elevated. In study by LaRue et al., transport of Aβ across the BBB was increased eight fold in amyloid transgenic mice compared to wild-type controls [109], a process that could be inhibited by immunization [12].

3.3. Dietary saturated fats and blood–brain delivery of lipoprotein associated amyloid-beta

The vasoactive properties of exogenous Aβ and in hyperamyloidic transgenic mice led us to explore the hypothesis that dietary SFA increases plasma TRL-Aβ and that with chronic ingestion this consequently leads to blood-to-brain delivery of TRL-Aβ. In a recent study, wild-type mice were fed diets modified diets enriched in either SFA, monounsaturated (MUFA) or poly-unsaturated (PUFA) fatty acids and compared with low-fat fed controls [106] (Fig. 2). Following 3 months of dietary intervention there was remarkable parenchymal colocalization of Aβ with apo B immunoreactivity in SFA-supplemented mice. Six months of SFA feeding increased immunoreactive Aβ/apo B compared to the 3 month fed group and the pattern of distribution was remarkably similar to Aβ/apo B colocalization in APP/PS1 amyloid transgenic mice with cortex > brain stem > hippocampal formation. However, there was no evidence that TRL-Aβ delivery to brain occurred in either MUFA, PUFA or low-fat fed mice.

A shift in receptor-mediated transport across the BBB may have contributed to extravasation of apo B-Aβ in SFA mice. However, several other markers suggest that was more likely to be a non-specific phenomenon. Immunoglobulin G (IgG), a large molecular weight plasma protein, was evident in parenchyma of SFA-fed mice and occludin expression, the primary endothelial tight junction protein was substantially reduced compared to controls. In addition, the plasma concentration of S100B, a CSF abundant protein, was increased in plasma suggesting bidirectional disturbances in protein transport across the BBB.

4. Saturated fatty acid induced disturbances in blood–brain barrier integrity

4.1. Triacylglycerol-rich-lipoprotein amyloid-beta-induced cerbrovascular disturbances

It is proposed that post-prandial hyperamyloidemia is one possible mechanism for SFA-induced BBB dysfunction and delivery of TRL-Aβ from blood-to-brain, but presently this remains to be substantiated. Rather, we found that the plasma concentration of $Aβ_{1-40}$ and $Aβ_{1-42}$ in SFA-fed mice was similar to mice maintained on either MUFA, PUFA or low-fat (control) diets [106] (Fig. 2). However, caution must be exercised with this interpretation. Post-prandial hyperamyloidemia may not have been apparent in those studies because the mice had been deprived of food for approximately 6 h before blood was sampled. Alternatively, repetitive but transient (post-meal) exposure to post-prandial-Aβ may be sufficiently damaging to endothelial integrity, without inducing a state of basal hyperamyloidemia. Consistent with this concept, in non-demented participants significant variation in CSF-Aβ levels of 1.5- to 4-fold were detected over 36 h of serial sampling. Amyloid-$β_{1-40}$ and $Aβ_{1-42}$ were highly correlated over time indicating that similar processes regulate the concentration of these isoforms. On average, the fluctuations of Aβ levels appeared to be time of day or activity dependent [110]. Methodological limitations may also be a confounder in interpretation. It is possible that the immunoassays used to measure plasma Aβ are not sensitive to the

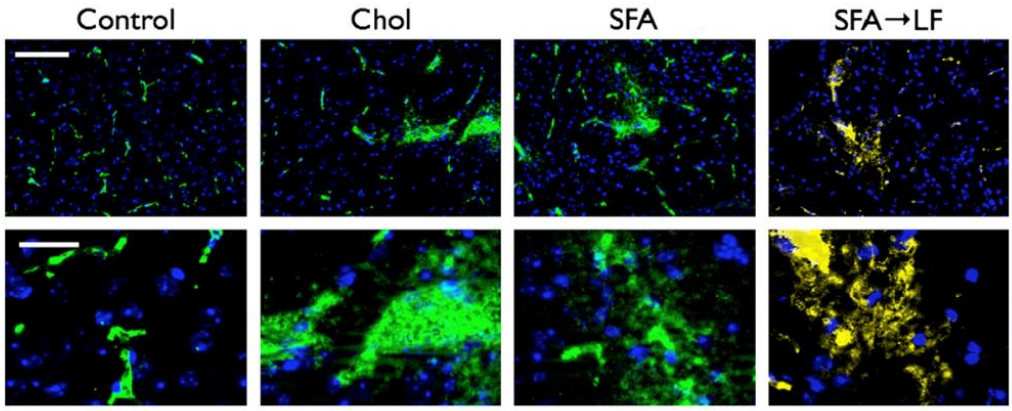


Fig. 2. Integrity of blood–brain barrier shown by the cerebral distribution of plasma immunoglobulin G. The cerebral distribution of immunoglobulin G (IgG) is shown in mice fed either a low-fat control, saturated fat enriched (SFA) or cholesterol (Chol) supplemented diet for 60 days. Significant peri-vascular leakage of IgG was seen in both SFA and cholesterol fed mice consistent with BBB dysfunction. Fourteen days after replacing the SFA diet with the low-fat diet (SFA → LF) IgG extravasation persisted. Scale bar in top and bottom row represents 100 μm and 30 μm, respectively.

lipidated form of Aβ and are unable to detect the putative SFA-induced increase in this pool of Aβ.

4.2. Saturated fatty acid induced amyloid-beta independent cerebrovacular disturbances

Several non-Aβ mediated pathways could also contribute to SFA-induced cerebrovascular disturbances. Dietary 'lipotoxicity' refers to the processes leading to end-organ damage and/or dysfunction following excess exposure to fatty acids identified in the context of fat-induced insulin resistance [111]. However, the process has also been implicated in endothelial dysfunction and atherosclerosis, heart failure, kidney failure, steatohepatitis and liver failure, autoimmune inflammatory disorders, susceptibility to infections, cancer and ageing. Significant differences in the cytotoxic effects of fatty acids have been reported, with longer chain SFA's being the most potent and the mono- and poly-unsaturated fatty acids being cytoprotective [112]. Morgan [112] suggests that the underlying toxicity of SFA is a consequence of disturbances in protein processing and endoplasmic reticulum dysfunction, for example apoptotic induction. One relevant example was a study by Patil et al. who found that palmitic acid induced region-specific cerebral damage because of higher fatty acid-metabolizing capacity of cortical astroglia [113]. Conversely, cell culture studies suggest that incubation, particularly with longer chain unsaturates has an antagonistic effect on endoplasmic reticulum-centred stress pathways [114]. If this were the case, then amyloidosis may be a phenomenon secondary to cerebrovascular inflammation.

Animal feeding studies have shown that typical Western diets substantially increase protein oxidation and lipid peroxidation [115,116]. In APP/PS1 mice, this occurred in the absence of increased Aβ levels [115]. In addition, differences in membrane lipid status as a consequence of diet may influence the propensity for Aβ oligomerization to occur [117]. Exogenous fatty acid supplementation results in significant shifts in neuronal phospholipids and in lipid raft composition [118–120], key regulators of cell protein transport and inflammation. Dietary fats also influence expression of critical genes involved in Aβ kinetics, for example the scavenger protein transthyretrin [77]. An alternate perspective is provided by Hooijmans and colleagues, who suggested that dietary fats influence AD risk because of chronic changes in cerebral hemodynamics [68]. In APP/PS1 mice fed DHA, plaque burden was attenuated probably because of greater blood circulation in the brain due to vasodilation. In contrast a Western diet rich in saturated fats and cholesterol increased amyloidoisis but without any changes to net blood volume or flow.

4.3. Cholesterol-induced disturbances in blood–brain barrier integrity

Studies by Ghbiri et al. found that like SFA, dietary cholesterol results in BBB dysfunction in New Zealand white rabbits [121]. Chronic dietary cholesterol supplementation also results in cerebral amyloidosis in wild-type rabbits, but this was not explored in the context of raised plasma TRL-Aβ [61]. However, indirect evidence that aberrant lipoprotein metabolism is involved in NZ-White rabbits fed cholesterol is suggested by the observation that the animals become grossly hypercholesterolemic as a consequence of apo B lipoprotein accumulation.

We have confirmed that modest dietary supplementation with cholesterol disturbs BBB function and, like SFA, extravasation of apo B/Aβ is observed within the brain parenchyma [106]. However, unlike the rabbit studies, mice were normolipaemic. Dietary cholesterol supplementation also had no measurable effect on plasma $A\beta_{1-40}$ or $A\beta_{1-42}$ in wild-type mice (albeit with the caveats in measurement discussed), consistent with the concept that the effects on BBB function were plasma Aβ independent. Cell culture studies suggest several mechanisms by which dietary cholesterol may be toxic. Frears et al. observed that, in the presence of cholesterol, human AβPP transfected HEK cells secrete greater quantities of Aβ [122]. However, the effects of cholesterol on Aβ biosynthesis are uncertain because cholesterol lowered Aβ synthesis in primary cell cultures of rat embryo hippocampal neurones [123] and dietary cholesterol reduces enterocytic abundance of Aβ [89]. Clearly, the effects of dietary cholesterol on net TRL-Aβ secretion in vivo need to be established. Alternatively, Subasinghe et al. showed that cholesterol can enhance Aβ induced toxicity because of increased protein binding to the plasma membrane and accelerated oligomerization of Aβ [124]. Yao and colleagues suggest that like SFA, excess cholesterol causes ER and mitochondrial stress that can lead to apoptosis [125,126]. Mitochondrial activity or lysosomal processing can result in the production of oxidized lipids including cholesterol. A number of studies support the contention that oxidized lipids compromise tissue integrity and exacerbate inflammatory pathways [127,128]. Interestingly, Stanyer and colleagues reported that plasma lipoproteins, particularly when oxidized, promote Aβ polymerization [129].

5. Apolipoprotein E phenotype, apo B-amyloid beta metabolism and Alzheimer's disease risk

5.1. Apolipoprotein E isoforms and Alzheimer's disease risk

Inheriting one or two alleles for apo E4 increase the risk of AD by 17% and 43%, respectively, compared to individuals hetero- or homo-zygous for apo E2 and E3 isoforms [130]. A number of hypotheses have been put forward for the positive association of AD with apo E4 and reviewed extensively in the literature [92,131–139]. Briefly, key concepts include; poorer sequestration of soluble Aβ and hence a propensity for oligomers to form; increased Aβ biosynthesis by regulating the activities of APP cleavage enzymes of beta- and gamma-secretase; disturbances in cholesterol homeostasis, which in turn will regulate Aβ biogenesis; pro-inflammatory and oxidative stress triggers; improper maintenance of BBB integrity; and defective neuronal growth. In this review, we wish to also consider the possibility that apo E isoforms influence AD risk via differential modulation of TRL metabolism.

5.2. Apolipoprotein E isoforms and triacylglycerol-rich-lipoprotein metabolism

More than 98% of plasma apo E exists in a lipidated form, principally bound to post-hydrolyzed TRL-remnants [140,141]. Apo E is the principal protein of chylomicrons, making up approximately 65% of total protein mass [142]. The acquisition by TRL of apo E inhibits interaction with endothelial lipases, serving instead as the binding ligand to high affinity receptors involved in TRL-remnant uptake [143–145]. The primary receptor responsible for TRL-remnant uptake is the low-density-lipoprotein receptor (LDL-r), however if this pathway becomes rate-limiting other high affinity processes such as lipoprotein–receptor-related protein one LRP1 may partially compensate.

In man, the three principal apo E isoforms are differentially distributed, probably because of differences in lipophilicity. Curiously, apo E4 is distributed with remnant lipoproteins that contain relatively more TAG (principally chylomicrons), whereas apo E2 and apo E3 tend to be primarily associate with hepatically derived TRL remnants, (i.e. intermediate density lipoproteins) [146].

There are several pathways by which apo E or specific variants may synergistically influence TRL-Aβ mediated AD risk. Firstly, apo E4 does not support proper BBB functionality compared to apo E2 and E3 [103] which may result in amplified blood-to-brain delivery of plasma proteins including TRL-Aβ. Apo E per se has significant affinity for extracellular matrices in particular the heparin sulphate proteoglycans (HSPG). Physiologically this is an important function as it facilitates interaction with proteins involved in receptor-mediated uptake. Binding of apo E to HSPG is an initial step in the localization of TRL-remnant to the surface of several different types of cells. Thereafter, the TRL-remnants are transported into the cell by receptor-mediated pathways, or by direct uptake of apoE-containing lipoprotein-HSPG complex [135]. Studies by Libeu et al. found that apo E has an HSPG-binding site highly complementary to heparin sulphate rich in *N*- and *O*-sulfo groups in the brain and liver [147].

The physiological effect of apo E variant on HSPG binding is difficult to predict although mutations in apo E have demonstrated potentially substantial differences in affinity [148,149], Arg-142 [150,151], Arg-145 [151], and Lys-146 [152]. However, the dissociation constant of equilibrium K_D of the principal apoE isoforms and glycosaminoglycans (GAGs) was found to be similar [153]. Collectively, apo E may mediate extracellular retention of TRL-Aβ if delivered from blood-to-brain but presently there is no clear evidence to suggest this would be exacerbated in individuals who express the apo E4 variant.

In atherosclerotic plaque, retention of apo B/E lipoproteins within the subendothelial space is considered the triggering event for monocyte infiltration. Activated macrophages are potently equipped with an array of receptors capable of internalizing TRL-Aβ [154]. Apo E serves as the principal lipoprotein binding ligand for many of these uptake pathways including the LDL-r and LRP1. Activated macrophages will secrete substantial quantities of apo E to enhance the efficiency of lipoprotein internalization [155]. Oxidative modification of lipoproteins may occur particularly if retention is prolonged. Modification, enables macrophage internalization by additional apo E mediated pathways, such as via the scavenger receptor [156] and the oxidized LDL receptor LOX-1 [157]. Evidence that apo E variants may influence the inflammatory pathway comes from primary cultures of macrophages. Macrophages expressing apo E4 enhanced atherosclerotic pathways compared to apo E3 macrophages, by promoting LDL-r mediated lipoprotein uptake [158]. Moreover, apo E4 was also found to be less efficient at conferring oxidative protection than apo E3. In another study the murine monocyte–macrophage cell line (RAW 264.7) was stably transfected to produce equal amounts of human apoE3 or apoE4. Following lipopolysaccharide stimulation, apoE4-macrophages showed higher and lower concentrations of tumour necrosis factor alpha (pro-inflammatory) and interleukin 10 (anti-inflammatory). In addition, increased expression of heme oxygenase-1 (a stress-induced anti-inflammatory protein) was observed in the apoE4-cells. The apoE4-macrophages also had an enhanced transactivation of the key redox sensitive transcription factor NF-κB.

A number of studies have shown that TRL-remnants are efficiently degraded by macrophages. If uptake occurs, a mitochondrial respiratory burst and lysosomal exocytosis results in the release of potent cytotoxic compounds such as superoxide, which compromise cellular integrity [159]. Proteinaceous deposits may be formed if cell death occurs and it is likely this exacerbates inflammatory pathways. The latter would suggest that amyloidosis is a secondary inflammatory trigger but pivotal to a subsequent cyclic phenomenon. Glial cell activation is the hallmark of inflammation in the brain [160]. Activated microglia produce inflammatory molecules such as cytokines, growth factors and complement proteins [161–163]. These mediators of inflammation in turn activate other cells to produce additional signalling molecules that further activate microglia in a positive feedback loop to perpetuate and amplify the inflammatory signalling cascade [164].

Apo E is an important ligand for binding of TRL-remnants to LRP1, a key endothelial junction protein thought to primarily facilitate cerebral efflux of Aβ. However, in cultured 293 cells, LRP1 had approximately equal affinity for apo E2/E3 and E4 [165], suggesting that cerebrovascular-mediated efflux of Aβ via LRP would not be unduly different in subjects with apo E4 alleles.

6. Apolipoprotein B/amyloid beta association with proteoglycans in a murine model of Alzheimer's disease

6.1. Apolipoprotein B association with agrin, perlecan, biglycan and decorin

Proteoglycans are major components of the extracellular matrices, comprised of one or more glycosaminoglycans chains covalently attached to a core protein [166]. Proteoglycans may serve as binding sites for receptors, or as mediators of cell adhesion, migration and proliferation [166]. Studies over the past decade suggest that proteoglycans, in particularly heparin sulfate proteo-

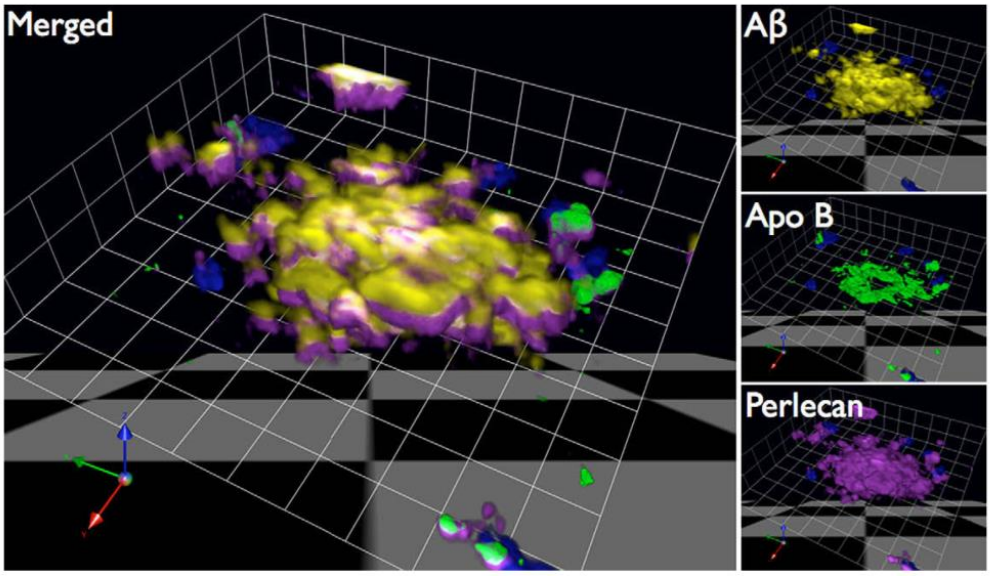


Fig. 3. The colocalization of apolipoprotein B, perlecan with cerebral amyloid plaques in amyloid transgenic mice. A 3-D triple immunofluorolabelling technique was utilized to investigate the colocalization of apolipoprotein B (apo B) and the proteoglycans perlecan, biglycan, agrin and decorin in the amyloid plaques of APP/PS1 transgenic amyloid mice. A representative image of apo B and perlecan colocalization is shown. There was significant colocalization of all four proteoglycans with amyloid plaque, but only perlecan, biglycan and decorin were positively associated with apo B lipoprotein retention (Lam, Takechi and Mamo unpublished observations). One unit of scale is indicative of 5 μm.

glycans, contribute towards the formation and thereafter stability of amyloid plaques [167]. However, their putative role in lipoprotein-Aβ entrapment has not been considered.

Proteoglycans bind apo E and apo B via ionic interactions with the core protein of proteoglycans [168,169] and proteoglycan mediated retention of apo B/E lipoproteins within the subendothelial space of arterial vessels is considered as the initiating event for atherosclerosis [170,171].

We suggest four proteoglycans that may be of particular importance to parenchymal binding of TRL-Aβ. Agrin is an extracellular matrix-associated HSPG pivotal for the development and the maintenance of the BBB and the formation of the neuromuscular junction [172]. Agrin exhibits structural similarity to perlecan, a proteoglycan reported to bind apo B lipoproteins in the hepatic sinusoidal space [173].

Perlecan, the largest extracellular matrix HSPG, has the capacity to facilitate the interaction of apo B and E lipoproteins with receptor-mediated pathways [173,174]. Perlecan exhibits structural homology to the ligand binding region of LDL-r, the primary pathway for apo B and apo E rich particle internalization [173,175]. Perlecan over-expression within the subendothelial space of coronary vessels has been implicated in the pathogenesis of atherosclerosis as a consequence of increased lipoprotein retention [176].

Cerebral biglycan expression in AD has not been reported. However, biglycan has significant affinity for apo B and E containing lipoproteins [177,178] and may contribute to the cerebral retention of TRL-Aβ. In atherosclerotic tissue, biglycan abundance is substantially greater in comparison to healthy tissue [179,180]. Moreover, there is a positive association between biglycan and arterial accumulation of apo B and E containing lipoproteins [177,178]. Decorin exhibits structural homology to biglycan (57%), including the apo B/E binding domain [173].

An immunhistological approach was used to investigate the putative colocalization of apo B/Aβ with agrin, perlecan, biglycan and decorin in an established murine model of AD. Double transgenic amyloid mice (APP/PS1) have an eightfold higher concentration of Aβ compared to wild-type mice and develop cerebral amyloid plaque by 6 months of age [105]. In APP/PS1 mice, focal accumulation of apo B lipoproteins was found with Aβ-plaque (Fig. 3). We found enrichment in cerebral amyloid deposits of the proteoglycans, agrin, perlecan, biglycan and decorin within the core of dense Aβ-plaque and an example of the perlecan/apo B/Aβ collocation is shown in Fig. 3. The Pearson's correlation coefficient was used as a measure of interdependent proteoglycan/apo B/Aβ association [181]. Of the four proteoglycans investigated, perlecan, biglycan and decorin were all positively associated with apo B lipoprotein abundance and with Aβ (Lam, Takechi and Mamo, unpublished data). These findings suggest that some proteoglycans contribute to Aβ retention and by extension amyloidosis, whilst other proteoglycans may have different functions, for example plaque stabilization [174,182,183].

7. Do hepatic and intestinally-derived apo B lipoproteins both contribute to Alzheimer's disease risk via increased blood-to-brain delivery and extracellular entrapment?

7.1. Apolipoprotein B isoforms and triacylglycerol-rich-lipoprotein kinetics

In man, hepatically derived TRL can be distinguished from chylomicrons based on the apo B_{100} and apo B_{48} isoforms respectively [184,185]. Apo B_{48} is synthesized in enterocytes as a consequence of mRNA processing and essentially represents half of the apo B_{100} amino acid sequence. It's not clear why this editing process occurs specifically in absorptive epithelial cells of the small intestine of man, suffice to say that this may be responsible for constitutive rates of chylomicron biogenesis in the absence of ingested fats.

Nascent TRL secreted from liver and intestine share similar metabolic pathways but there are some significant differences in metabolism which may be important in understanding AD risk. Chylomicrons and very low density lipoprotein (VLDL) interact

with endothelial lipases and become progressively depleted in TAG. The apo E rich post-hydrolyzed remnants then bind to receptors responsible for internalization. Chylomicrons are generally larger than VLDL and contain more TAG, yet hydrolysis to the remnant form is quicker. Once in circulation, chylomicron lipolysis and clearance is generally complete within about 15 min. Hydrolysis of VLDL TAG may take up several hours and approximately half of the VLDL-remnants will persist in circulation to become cholesterol rich (and apo E poor) LDL.

Several, but not all clinical studies suggest that fasting plasma apo B, which is primarily indicative of hepatically-derived lipoproteins, may be increased in subjects with AD [186]. Thus far only one study reported apo B_{48} in AD/MCI subjects and this was found to be substantially increased in the post-absorptive state [85]. Whilst the latter is consistent with post-prandial chylomicronemia, classical oral fat challenge tests have not yet been reported in AD/MCI subjects.

Unlike man, the liver of mice primarily secretes apo B_{48} and so there is no clearly distinguishing difference per se between lipoproteins of intestinal or hepatic origin in this species. Preliminary studies suggest that SFA supplementation in wild-type mice does not have the same stimulatory effect on Aβ and apo B abundance in hepatocytes as that observed for enterocytes (Galloway and Mamo, unpublished observations). This finding suggests that whilst the BBB disturbances reported in wild-type mice because of SFA feeding was specifically a post-prandial phenomenon, it does not rule out a role for hepatically-derived lipoproteins per se. Presently, there is no rationale to suggest that elevated apoB_{100} lipoprotein-Aβ would be any less challenging to cerebrovascular integrity than apo B_{48} lipoprotein-Aβ. A fundamental question then is whether extracellular entrapment by proteoglycans of apo B_{100} lipoproteins substantially differs from apo B_{48} lipoproteins. Both isoforms of apo B bind to heparin proteoglycans with significant affinity however the amino acid residues responsible differ substantially for the two isoforms [168,171]. Subtle differences in lipid composition can profoundly affect lipoprotein interaction with receptors and extracellular matrices so it is impossible to unequivocally say if there is a generic difference between apo B lipoproteins of hepatic and intestinal origin. In human atherosclerotic plaque both apo B_{100} and apo B_{48} are found [28,175], however there seems to be substantially more apo B_{48} than apo B_{100} relative to the plasma concentration of the two lipoprotein subtypes [187]. In LDL-r deficient rabbits that have massively elevated levels of apo B_{100} and apo B_{48} lipoprotein, only the latter was significantly increased in atherosclerotic plaque compared to healthy arterial tissue [188]. On the other hand, over-expression of human apo B (excluding brain) induces severe neurodegeneration in transgenic mice concomitant with elevated plasma TAG and Aβ deposition [189]. Collectively, there is some evidence to suggest that intestinally-derived apo B lipoproteins may be more prone to extracellular retention. However, their concentration in blood is typically much less than that of hepatically derived apo B lipoproteins. Therefore, information about the relative distribution of apo B isoforms in brain parenchyma and amyloid plaque would be informative.

8. Conclusion

The critical observations considered in this review are that dietary saturated fats and cholesterol cause BBB dysfunction, resulting in the blood-to-brain delivery of apo B lipoprotein-Aβ. In some individuals, dietary-induced disturbances in BBB integrity may be the initiating event for AD. If cerebrovascular disturbances are central to AD aetiology and progression, then considering strategies to positively influence integrity is a therapeutic priority. Presently, drug strategies used to treat AD are focussed on maintaining cell-cell communication rather than cerebrovascular function.

Some, but not all, clinical studies suggest that statins may reduce AD risk and progression [190–192] although the mechanisms for this putative effect are unclear. Relevant to the focus of this review, possibilities include reduced TRL-Aβ secretion; enhanced clearance from blood of TRL-remnants containing Aβ; maintenance of BBB function and anti-inflammatory properties. Fibrates can profoundly reduce TRL-secretion, but their efficacy in the context of BBB function and AD risk has not been considered.

Understanding the mechanisms by which dietary fats influence AD risk reinforces and substantiates the good nutrition public health strategies for prevention of disease. In a treatment context there may also be substantial value in knowing these mechanisms. However, developing nutritional/lifestyle or drugs which potentially may confer cerebrovascular benefit is not likely to be useful unless environmental and endogenous cerebrovascular 'insults' are synergistically considered.

References

[1] Joachim CL, Duffy LK, Morris JH, Selkoe DJ. Protein chemical and immunocytochemical studies of meningovascular beta-amyloid protein in Alzheimer's disease and normal aging. Brain Res 1988;474:100–11.
[2] Joachim CL, Morris JH, Selkoe DJ. Clinically diagnosed Alzheimer's disease: autopsy results in 150 cases. Ann Neurol 1988;24:50–6.
[3] Duyckaerts C, Delatour B, Potier MC. Classification and basic pathology of Alzheimer disease. Acta Neuropathol 2009;118:5–36.
[4] Selkoe DJ. Alzheimer's disease is a synaptic failure. Science 2002;298:789–91.
[5] Pimplikar SW. Reassessing the amyloid cascade hypothesis of Alzheimer's disease. Int J Biochem Cell Biol 2009;41:1261–8.
[6] Kern A, Behl C. The unsolved relationship of brain aging and late-onset Alzheimer disease. Biochim Biophys Acta 2009;1790:1124–32.
[7] Tomita T, Tokuhiro S, Hashimoto T, Aiba K, Saido TC, Maruyama K, et al. Molecular dissection of domains in mutant presenilin 2 that mediate overproduction of amyloidogenic forms of amyloid beta peptides. Inability of truncated forms of PS2 with familial Alzheimer's disease mutation to increase secretion of Abeta42. J Biol Chem 1998;273:21153–60.
[8] Citron M, Westaway D, Xia W, Carlson G, Diehl T, Levesque G, et al. Mutant presenilins of Alzheimer's disease increase production of 42-residue amyloid beta-protein in both transfected cells and transgenic mice. Nat Med 1997;3:67–72.
[9] Kirkitadze MD, Kowalska A. Molecular mechanisms initiating amyloid beta-fibril formation in Alzheimer's disease. Acta Biochim Pol 2005;52:417–23.
[10] Hardy JA, Higgins GA. Alzheimer's disease: the amyloid cascade hypothesis. Science 1992;256:184–5.
[11] Cummings JL, Vinters HV, Cole GM, Khachaturian ZS. Alzheimer's disease: etiologies, pathophysiology, cognitive reserve, and treatment opportunities. Neurology 1998;51:S2–17 [Discussion S65–S17].
[12] Deane R, Sagare A, Hamm K, Parisi M, LaRue B, Guo H, et al. IgG-assisted age-dependent clearance of Alzheimer's amyloid beta peptide by the blood–brain barrier neonatal Fc receptor. J Neurosci 2005;25:11495–503.
[13] Deane R, Bell RD, Sagare A, Zlokovic BV. Clearance of amyloid-beta peptide across the blood–brain barrier: implication for therapies in Alzheimer's disease. CNS Neurol Disord Drug Targets 2009;8:16–30.
[14] Crossgrove JS, Li GJ, Zheng W. The choroid plexus removes beta-amyloid from brain cerebrospinal fluid. Exp Biol Med (Maywood) 2005;230:771–6.
[15] Strazielle N, Ghersi-Egea JF. Choroid plexus in the central nervous system: biology and physiopathology. J Neuropathol Exp Neurol 2000;59:561–74.
[16] Deane R, Du Yan S, Submamaryan RK, LaRue B, Jovanovic S, Hogg E, et al. RAGE mediates amyloid-beta peptide transport across the blood–brain barrier and accumulation in brain. Nat Med 2003;9:907–13.
[17] Donahue JE, Flaherty SL, Johanson CE, Duncan 3rd JA, Silverberg GD, Miller MC, et al. RAGE, LRP-1, and amyloid-beta protein in Alzheimer's disease. Acta Neuropathol 2006;112:405–15.
[18] Zlokovic BV, Ghiso J, Mackic JB, McComb JG, Weiss MH, Frangione B. Blood–brain barrier transport of circulating Alzheimer's amyloid beta. Biochem Biophys Res Commun 1993;197:1034–40.
[19] Martel CL, Mackic JB, McComb JG, Ghiso J, Zlokovic BV. Blood–brain barrier uptake of the 40 and 42 amino acid sequences of circulating Alzheimer's amyloid beta in guinea pigs. Neurosci Lett 1996;206:157–60.
[20] Maness LM, Banks WA, Podlisny MB, Selkoe DJ, Kastin AJ. Passage of human amyloid beta-protein 1–40 across the murine blood–brain barrier. Life Sci 1994;55:1643–50.
[21] de la Torre JC. Cerebrovascular and cardiovascular pathology in Alzheimer's disease. Int Rev Neurobiol 2009;84:35–48.
[22] Viswanathan A, Rocca WA, Tzourio C. Vascular risk factors and dementia: how to move forward? Neurology 2009;72:368–74.
[23] Duron E, Hanon O. Vascular risk factors, cognitive decline, and dementia. Vasc Health Risk Manag 2008;4:363–81.

[24] Rosendorff C, Beeri MS, Silverman JM. Cardiovascular risk factors for Alzheimer's disease. Am J Geriatr Cardiol 2007;16:143–9.
[25] Rosano C, Newman AB. Cardiovascular disease and risk of Alzheimer's disease. Neurol Res 2006;28:612–20.
[26] Meyer E, Westerveld HT, de Ruyter-Meijstek FC, van Greevenbroek MM, Rienks R, van Rijn HJ, et al. Abnormal postprandial apolipoprotein B-48 and triglyceride responses in normolipidemic women with greater than 70% stenotic coronary artery disease: a case-control study. Atherosclerosis 1996;124:221–35.
[27] Phillips NR, Waters D, Havel RJ. Plasma lipoproteins and progression of coronary artery disease evaluated by angiography and clinical events. Circulation 1993;88:2762–70.
[28] Proctor SD, Vine DF, Mamo JC. Arterial retention of apolipoprotein B(48)- and B(100)-containing lipoproteins in atherogenesis. Curr Opin Lipidol 2002;13:461–70.
[29] Fishbein GA, Fishbein MC. Arteriosclerosis: rethinking the current classification. Arch Pathol Lab Med 2009;133:1309–16.
[30] Weuve J, Kang JH, Manson JE, Breteler MM, Ware JH, Grodstein F. Physical activity, including walking, and cognitive function in older women. JAMA 2004;292:1454–61.
[31] Abbott RD, White LR, Ross GW, Masaki KH, Curb JD, Petrovitch H. Walking and dementia in physically capable elderly men. JAMA 2004;292:1447–53.
[32] Larson EB, Wang L, Bowen JD, McCormick WC, Teri L, Crane P, et al. Exercise is associated with reduced risk for incident dementia among persons 65 years of age and older. Ann Intern Med 2006;144:73–81.
[33] Podewils LJ, Guallar E, Kuller LH, Fried LP, Lopez OL, Carlson M, et al. Physical activity, APOE genotype, and dementia risk: findings from the cardiovascular health cognition study. Am J Epidemiol 2005;161:639–51.
[34] van Gelder BM, Tijhuis MA, Kalmijn S, Giampaoli S, Nissinen A, Kromhout D. Physical activity in relation to cognitive decline in elderly men: the FINE study. Neurology 2004;63:2316–21.
[35] Santana-Sosa E, Barriopedro MI, Lopez-Mojares LM, Perez M, Lucia A. Exercise training is beneficial for Alzheimer's patients. Int J Sports Med 2008;29:845–50.
[36] Kivipelto M, Solomon A. Alzheimer's disease – the ways of prevention. J Nutr Health Aging 2008;12:89S–94S.
[37] Pasinetti GM, Eberstein JA. Metabolic syndrome and the role of dietary lifestyles in Alzheimer's disease. J Neurochem 2008;106:1503–14.
[38] Brookmeyer R, Johnson E, Ziegler-Graham K, Arrighi HM. Forecasting the global burden of Alzheimer's disease. Alzheimers Demen 2007;3:186–91.
[39] Bickel H. Dementia in advanced age: estimating incidence and health care costs. Z Gerontol Geriatr 2001;34:108–15.
[40] Guest Editors 2008 Alzheimer's disease facts and figures. Alzheimers Demen 2008;4:110–133.
[41] Ellis RJ, Olichney JM, Thal LJ, Mirra SS, Morris JC, Beekly D, et al. Cerebral amyloid angiopathy in the brains of patients with Alzheimer's disease: the CERAD experience, Part XV. Neurology 1996;46:1592–6.
[42] Wisniewski HM, Vorbrodt AW, Wegiel J. Amyloid angiopathy and blood–brain barrier changes in Alzheimer's disease. Ann NY Acad Sci 1997;826:161–72.
[43] Kalaria RN. The blood–brain barrier and cerebral microcirculation in Alzheimer disease. Cerebrovasc Brain Metab Rev 1992;4:226–60.
[44] Cullen KM. Perivascular astrocytes within Alzheimer's disease plaques. Neuroreport 1997;8:1961–6.
[45] Itagaki S, McGeer PL, Akiyama H, Zhu S, Selkoe D. Relationship of microglia and astrocytes to amyloid deposits of Alzheimer disease. J Neuroimmunol 1989;24:173–82.
[46] Barberger-Gateau P, Raffaitin C, Letenneur L, Berr C, Tzourio C, Dartigues JF, et al. Dietary patterns and risk of dementia: the three-city cohort study. Neurology 2007;69:1921–30.
[47] Oba S, Nagata C, Nakamura K, Fujii K, Kawachi T, Takatsuka N, et al. Diet based on the Japanese food guide spinning top and subsequent mortality among men and women in a general Japanese population. J Am Diet Assoc 2009;109:1540–7.
[48] Yusuf S, Hawken S, Ounpuu S, Dans T, Avezum A, Lanas F, et al. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. Lancet 2004;364:937–52.
[49] Menotti A, Kromhout D, Blackburn H, Fidanza F, Buzina R, Nissinen A. Food intake patterns and 25-year mortality from coronary heart disease: cross-cultural correlations in the seven countries study. The seven countries study research group. Eur J Epidemiol 1999;15:507–15.
[50] Singh RB, Dubnov G, Niaz MA, Ghosh S, Singh R, Rastogi SS, et al. Effect of an Indo-Mediterranean diet on progression of coronary artery disease in high risk patients (Indo-Mediterranean diet heart study): a randomised single-blind trial. Lancet 2002;360:1455–61.
[51] Vincent-Baudry S, Defoort C, Gerber M, Bernard MC, Verger P, Helal O, et al. The Medi-RIVAGE study: reduction of cardiovascular disease risk factors after a 3-mo intervention with a Mediterranean-type diet or a low-fat diet. Am J Clin Nutr 2005;82:964–71.
[52] De Lorgeril M, Salen P, Martin JL, Mamelle N, Monjaud I, Touboul P, et al. Effect of a mediterranean type of diet on the rate of cardiovascular complications in patients with coronary artery disease. Insights into the cardioprotective effect of certain nutriments. J Am Coll Cardiol 1996;28:1103–8.
[53] de Lorgeril M, Salen P, Martin JL, Monjaud I, Delaye J, Mamelle N. Mediterranean diet, traditional risk factors, and the rate of cardiovascular complications after myocardial infarction: final report of the Lyon diet heart study. Circulation 1999;99:779–85.
[54] Engelhart MJ, Geerlings MI, Ruitenberg A, Van Swieten JC, Hofman A, Witteman JC, et al. Diet and risk of dementia: does fat matter? The Rotterdam study. Neurology 2002;59:1915–21.
[55] Morris MC, Evans DA, Bienias JL, Tangney CC, Bennett DA, Aggarwal N, et al. Dietary fats and the risk of incident Alzheimer disease. Arch Neurol 2003;60:194–200.
[56] Kalmijn S. Fatty acid intake and the risk of dementia and cognitive decline: a review of clinical and epidemiological studies. J Nutr Health Aging 2000;4:202–7.
[57] Luchsinger JA, Mayeux R. Dietary factors and Alzheimer's disease. Lancet Neurol 2004;3:579–87.
[58] Solfrizzi V, D'Introno A, Colacicco AM, Capurso C, Del Parigi A, Capurso S, et al. Dietary fatty acids intake: possible role in cognitive decline and dementia. Exp Gerontol 2005;40:257–70.
[59] Solfrizzi V, Frisardi V, Capurso C, D'Introno A, Colacicco AM, Vendemiale G, et al. Dietary fatty acids in dementia and predementia syndromes: epidemiological evidence and possible underlying mechanisms. Ageing Res Rev 2009.
[60] Laitinen MH, Ngandu T, Rovio S, Helkala EL, Uusitalo U, Viitanen M, et al. Fat intake at midlife and risk of dementia and Alzheimer's disease: a population-based study. Dement Geriatr Cogn Disord 2006;22:99–107.
[61] Sparks DL, Scheff SW, Hunsaker 3rd JC, Liu H, Landers T, Gross DR. Induction of Alzheimer-like beta-amyloid immunoreactivity in the brains of rabbits with dietary cholesterol. Exp Neurol 1994;126:88–94.
[62] Oksman M, Iivonen H, Hogyes E, Amtul Z, Penke B, Leenders I, et al. Impact of different saturated fatty acid, polyunsaturated fatty acid and cholesterol containing diets on beta-amyloid accumulation in APP/PS1 transgenic mice. Neurobiol Dis 2006;23:563–72.
[63] Refolo LM, Malester B, LaFrancois J, Bryant-Thomas T, Wang R, Tint GS, et al. Hypercholesterolemia accelerates the Alzheimer's amyloid pathology in a transgenic mouse model. Neurobiol Dis 2000;7:321–31.
[64] Shie FS, Jin LW, Cook DG, Leverenz JB, LeBoeuf RC. Diet-induced hypercholesterolemia enhances brain a beta accumulation in transgenic mice. Neuroreport 2002;13:455–9.
[65] Schaefer EJ, Bongard V, Beiser AS, Lamon-Fava S, Robins SJ, Au R, et al. Plasma phosphatidylcholine docosahexaenoic acid content and risk of dementia and Alzheimer disease: the Framingham heart study. Arch Neurol 2006;63:1545–50.
[66] Hooijmans CR, Van der Zee CE, Dederen PJ, Brouwer KM, Reijmer YD, van Groen T, et al. DHA and cholesterol containing diets influence Alzheimer-like pathology, cognition and cerebral vasculature in APPswe/PS1dE9 mice. Neurobiol Dis 2009;33:482–98.
[67] Lim GP, Calon F, Morihara T, Yang F, Teter B, Ubeda O, et al. A diet enriched with the omega-3 fatty acid docosahexaenoic acid reduces amyloid burden in an aged Alzheimer mouse model. J Neurosci 2005;25:3032–40.
[68] Hooijmans CR, Rutters F, Dederen PJ, Gambarota G, Veltien A, van Groen T, et al. Changes in cerebral blood volume and amyloid pathology in aged Alzheimer APP/PS1 mice on a docosahexaenoic acid (DHA) diet or cholesterol enriched typical western diet (TWD). Neurobiol Dis 2007;28:16–29.
[69] Cole GM, Frautschy SA. Docosahexaenoic acid protects from amyloid and dendritic pathology in an Alzheimer's disease mouse model. Nutr Health 2006;18:249–59.
[70] Scarmeas N, Stern Y, Mayeux R, Manly JJ, Schupf N, Luchsinger JA. Mediterranean diet and mild cognitive impairment. Arch Neurol 2009;66:216–25.
[71] Petot GJ, Friedland RP. Lipids, diet and Alzheimer disease: an extended summary. J Neurol Sci 2004;226:31–3.
[72] Wu A, Ying Z, Gomez-Pinilla F. Docosahexaenoic acid dietary supplementation enhances the effects of exercise on synaptic plasticity and cognition. Neuroscience 2008;155:751–9.
[73] Cunnane SC, Plourde M, Pifferi F, Begin M, Feart C, Barberger-Gateau P. Fish, docosahexaenoic acid and Alzheimer's disease. Prog Lipid Res 2009;48:239–56.
[74] Arendash GW, Jensen MT, Salem Jr N, Hussein N, Cracchiolo J, Dickson A, et al. A diet high in omega-3 fatty acids does not improve or protect cognitive performance in Alzheimer's transgenic mice. Neuroscience 2007;149:286–302.
[75] Kaduce TL, Chen Y, Hell JW, Spector AA. Docosahexaenoic acid synthesis from n-3 fatty acid precursors in rat hippocampal neurons. J Neurochem 2008;105:1525–35.
[76] DeWille JW, Farmer SJ. Postnatal dietary fat influences mRNAS involved in myelination. Dev Neurosci 1992;14:61–8.
[77] Puskas LG, Kitajka K, Nyakas C, Barcelo-Coblijn G, Farkas T. Short-term administration of omega 3 fatty acids from fish oil results in increased transthyretin transcription in old rat hippocampus. Proc Natl Acad Sci USA 2003;100:1580–5.
[78] Sarsilmaz M, Songur A, Ozyurt H, Kus I, Ozen OA, Ozyurt B, et al. Potential role of dietary omega-3 essential fatty acids on some oxidant/antioxidant parameters in rats' corpus striatum. Prostaglandins Leukot Essent Fatty Acids 2003;69:253–9.
[79] Serhan CN, Yacoubian S, Yang R. Anti-inflammatory and proresolving lipid mediators. Annu Rev Pathol 2008;3:279–312.

[80] Hashimoto M, Hossain S, Shimada T, Sugioka K, Yamasaki H, Fujii Y, et al. Docosahexaenoic acid provides protection from impairment of learning ability in Alzheimer's disease model rats. J Neurochem 2002;81:1084–91.
[81] Koudinov AR, Koudinova NV, Kumar A, Beavis RC, Ghiso J. Biochemical characterization of Alzheimer's soluble amyloid beta protein in human cerebrospinal fluid: association with high density lipoproteins. Biochem Biophys Res Commun 1996;223:592–7.
[82] James AP, Mamo JC. The immunodetection of lipoprotein-bound amyloid-beta is attenuated because of the presence of lipids. Ann Clin Biochem 2005;42:70–2.
[83] Yanagisawa K, McLaurin J, Michikawa M, Chakrabartty A, Ihara Y. Amyloid beta-protein (A beta) associated with lipid molecules: immunoreactivity distinct from that of soluble A beta. FEBS Lett 1997;420:43–6.
[84] Biere AL, Ostaszewski B, Stimson ER, Hyman BT, Maggio JE, Selkoe DJ. Amyloid beta-peptide is transported on lipoproteins and albumin in human plasma. J Biol Chem 1996;271:32916–22.
[85] Mamo JC, Jian L, James AP, Flicker L, Esselmann H, Wiltfang J. Plasma lipoprotein beta-amyloid in subjects with Alzheimer's disease or mild cognitive impairment. Ann Clin Biochem 2008;45:395–403.
[86] Koudinov AR, Koudinova NV. Alzheimer's soluble amyloid beta protein is secreted by HepG2 cells as an apolipoprotein. Cell Biol Int 1997;21:265–71.
[87] Galloway S, Jian L, Johnsen R, Chew S, Mamo JC. Beta-amyloid or its precursor protein is found in epithelial cells of the small intestine and is stimulated by high-fat feeding. J Nutr Biochem 2007;18:279–84.
[88] Galloway S, Pallebage-Gamarallage MM, Takechi R, Jian L, Johnsen RD, Dhaliwal SS, et al. Synergistic effects of high fat feeding and apolipoprotein E deletion on enterocytic amyloid-beta abundance. Lipids Health Dis 2008;7:15.
[89] Pallebage-Gamarallage MM, Galloway S, Johnsen R, Jian L, Dhaliwal S, Mamo JC. The effect of exogenous cholesterol and lipid-modulating agents on enterocytic amyloid-beta abundance. Br J Nutr 2009;101:340–7.
[90] Takechi R, Galloway S, Pallebage-Gamarallage MM, Mamo JC. Chylomicron amyloid-beta in the aetiology of Alzheimer's disease. Atheroscler Suppl 2008;9:19–25.
[91] Takechi R, Galloway S, Pallebage-Gamarallage MM, Johnsen RD, Mamo JC. Three-dimensional immunofluorescent double labelling using polyclonal antibodies derived from the same species: enterocytic colocalization of chylomicrons with Golgi apparatus. Histochem Cell Biol 2008;129:779–84.
[92] Boyt AA, Taddei K, Hallmayer J, Mamo J, Helmerhorst E, Gandy SE, et al. Relationship between lipid metabolism and amyloid precursor protein and apolipoprotein E. Alzheimer's Rep 1999;2:339–46.
[93] James AP, Pal S, Gennat HC, Vine DF, Mamo JC. The incorporation and metabolism of amyloid-beta into chylomicron-like lipid emulsions. J Alzheimers Dis 2003;5:179–88.
[94] Jackson KG, Maitin V, Leake DS, Yaqoob P, Williams CM. Saturated fat-induced changes in Sf 60–400 particle composition reduces uptake of LDL by HepG2 cells. J Lipid Res 2006;47:393–403.
[95] Roberts CK, Barnard RJ, Liang KH, Vaziri ND. Effect of diet on adipose tissue and skeletal muscle VLDL receptor and LPL: implications for obesity and hyperlipidemia. Atherosclerosis 2002;161:133–41.
[96] Hayes KC, Khosla P, Hajri T, Pronczuk A. Saturated fatty acids and LDL receptor modulation in humans and monkeys. Prostaglandins Leukot Essent Fatty Acids 1997;57:411–8.
[97] Goldberg IJ. Hypertriglyceridemia: impact and treatment. Endocrinol Metab Clin North Am 2009;38:137–49.
[98] Karpe F, Hellenius ML, Hamsten A. Differences in postprandial concentrations of very-low-density lipoprotein and chylomicron remnants between normotriglyceridemic and hypertriglyceridemic men with and without coronary heart disease. Metabolism 1999;48:301–7.
[99] Proctor SD, Mamo JC. Arterial fatty lesions have increased uptake of chylomicron remnants but not low-density lipoproteins. Coron Artery Dis 1996;7:239–45.
[100] Mamo JC, Proctor SD, Smith D. Retention of chylomicron remnants by arterial tissue; importance of an efficient clearance mechanism from plasma. Atherosclerosis 1998;141(Suppl. 1):S63–9.
[101] Burgess BL, McIsaac SA, Naus KE, Chan JY, Tansley GH, Yang J, et al. Elevated plasma triglyceride levels precede amyloid deposition in Alzheimer's disease mouse models with abundant A beta in plasma. Neurobiol Dis 2006;24:114–27.
[102] Namba Y, Tsuchiya H, Ikeda K. Apolipoprotein B immunoreactivity in senile plaque and vascular amyloids and neurofibrillary tangles in the brains of patients with Alzheimer's disease. Neurosci Lett 1992;134:264–6.
[103] Deane R, Sagare A, Hamm K, Parisi M, Lane S, Finn MB, et al. ApoE isoform-specific disruption of amyloid beta peptide clearance from mouse brain. J Clin Invest 2008;118:4002–13.
[104] Ye S, Huang Y, Mullendorff K, Dong L, Giedt G, Meng EC, et al. Apolipoprotein (apo) E4 enhances amyloid beta peptide production in cultured neuronal cells: apoE structure as a potential therapeutic target. Proc Natl Acad Sci USA 2005;102:18700–5.
[105] Takechi R, Galloway S, Pallebage-Gamarallage M, Wellington C, Johnsen R, Mamo JC. Three-dimensional colocalization analysis of plasma-derived apolipoprotein B with amyloid plaques in APP/PS1 transgenic mice. Histochem Cell Biol 2009;131:661–6.
[106] Takechi R, Galloway S, Pallebage-Gamarallage M, Wellington C, Johnsen R, Dahliwal S, et al. Differential effects of dietary fatty acids on the cerebral distribution of plasma derived apo B lipoproteins with amyloid-beta. Br J Nutr 2009;28:1–11. PMID: 19860996. [Epub ahead of print].
[107] Thomas T, McLendon C, Sutton ET, Thomas G. Cerebrovascular endothelial dysfunction mediated by beta-amyloid. Neuroreport 1997;8:1387–91.
[108] George AJ, Holsinger RM, McLean CA, Laughton KM, Beyreuther K, Evin G, et al. APP intracellular domain is increased and soluble Abeta is reduced with diet-induced hypercholesterolemia in a transgenic mouse model of Alzheimer disease. Neurobiol Dis 2004;16:124–32.
[109] LaRue B, Hogg E, Sagare A, Jovanovic S, Maness L, Maurer C, et al. Method for measurement of the blood–brain barrier permeability in the perfused mouse brain: application to amyloid-beta peptide in wild type and Alzheimer's Tg2576 mice. J Neurosci Methods 2004;138:233–42.
[110] Bateman RJ, Wen G, Morris JC, Holtzman DM. Fluctuations of CSF amyloid-beta levels: implications for a diagnostic and therapeutic biomarker. Neurology 2007;68:666–9.
[111] Unger RH. Lipotoxicity in the pathogenesis of obesity-dependent NIDDM. Genetic and clinical implications. Diabetes 1995;44:863–70.
[112] Morgan NG. Fatty acids and beta-cell toxicity. Curr Opin Clin Nutr Metab Care 2009;12:117–22.
[113] Patil S, Balu D, Melrose J, Chan C. Brain region-specificity of palmitic acid-induced abnormalities associated with Alzheimer's disease. BMC Res Notes 2008;1:20.
[114] Diakogiannaki E, Morgan NG. Differential regulation of the ER stress response by long-chain fatty acids in the pancreatic beta-cell. Biochem Soc Trans 2008;36:959–62.
[115] Studzinski CM, Li F, Bruce-Keller AJ, Fernandez-Kim SO, Zhang L, Weidner AM, et al. Effects of short-term Western diet on cerebral oxidative stress and diabetes related factors in APP x PS1 knock-in mice. J Neurochem 2009;108:860–6.
[116] Ronti T, Lupattelli G, Mannarino E. The endocrine function of adipose tissue: an update. Clin Endocrinol (Oxf) 2006;64:355–65.
[117] Florent-Bechard S, Desbene C, Garcia P, Allouche A, Youssef I, Escanye MC, et al. The essential role of lipids in Alzheimer's disease. Biochimie 2009;91:804–9.
[118] Wassall SR, Stillwell W. Polyunsaturated fatty acid-cholesterol interactions: domain formation in membranes. Biochim Biophys Acta 2009;1788:24–32.
[119] Wassall SR, Brzustowicz MR, Shaikh SR, Cherezov V, Caffrey M, Stillwell W. Order from disorder, corralling cholesterol with chaotic lipids. The role of polyunsaturated lipids in membrane raft formation. Chem Phys Lipids 2004;132:79–88.
[120] Diaz O, Berquand A, Dubois M, Di Agostino S, Sette C, Bourgoin S, et al. The mechanism of docosahexaenoic acid-induced phospholipase D activation in human lymphocytes involves exclusion of the enzyme from lipid rafts. J Biol Chem 2002;277:39368–78.
[121] Ghribi O, Golovko MY, Larsen B, Schrag M, Murphy EJ. Deposition of iron and beta-amyloid plaques is associated with cortical cellular damage in rabbits fed with long-term cholesterol-enriched diets. J Neurochem 2006;99:438–49.
[122] Frears ER, Stephens DJ, Walters CE, Davies H, Austen BM. The role of cholesterol in the biosynthesis of beta-amyloid. Neuroreport 1999;10:1699–705.
[123] Abad-Rodriguez J, Ledesma MD, Craessaerts K, Perga S, Medina M, Delacourte A, et al. Neuronal membrane cholesterol loss enhances amyloid peptide generation. J Cell Biol 2004;167:953–60.
[124] Subasinghe S, Unabia S, Barrow CJ, Mok SS, Aguilar MI, Small DH. Cholesterol is necessary both for the toxic effect of Abeta peptides on vascular smooth muscle cells and for Abeta binding to vascular smooth muscle cell membranes. J Neurochem 2003;84:471–9.
[125] Feng B, Yao PM, Li Y, Devlin CM, Zhang D, Harding HP, et al. The endoplasmic reticulum is the site of cholesterol-induced cytotoxicity in macrophages. Nat Cell Biol 2003;5:781–92.
[126] Yao PM, Tabas I. Free cholesterol loading of macrophages is associated with widespread mitochondrial dysfunction and activation of the mitochondrial apoptosis pathway. J Biol Chem 2001;276:42468–76.
[127] Clare K, Hardwick SJ, Carpenter KL, Weeratunge N, Mitchinson MJ. Toxicity of oxysterols to human monocyte-macrophages. Atherosclerosis 1995;118:67–75.
[128] Peng SK, Tham P, Taylor CB, Mikkelson B. Cytotoxicity of oxidation derivatives of cholesterol on cultured aortic smooth muscle cells and their effect on cholesterol biosynthesis. Am J Clin Nutr 1979;32:1033–42.
[129] Stanyer L, Betteridge DJ, Smith CC. An investigation into the mechanisms mediating plasma lipoprotein-potentiated beta-amyloid fibrillogenesis. FEBS Lett 2002;518:72–8.
[130] Strittmatter WJ, Roses AD. Apolipoprotein E and Alzheimer's disease. Annu Rev Neurosci 1996;19:53–77.
[131] Donahue JE, Johanson CE, Apolipoprotein E. Amyloid-beta, and blood–brain barrier permeability in Alzheimer disease. J Neuropathol Exp Neurol 2008;67:261–70.
[132] Fryer JD, Simmons K, Parsadanian M, Bales KR, Paul SM, Sullivan PM, et al. Human apolipoprotein E4 alters the amyloid-beta 40:42 ratio and promotes the formation of cerebral amyloid angiopathy in an amyloid precursor protein transgenic model. J Neurosci 2005;25:2803–10.
[133] Irizarry MC, Deng A, Lleo A, Berezovska O, Von Arnim CA, Martin-Rehrmann M, et al. Apolipoprotein E modulates gamma-secretase cleavage of the amyloid precursor protein. J Neurochem 2004;90:1132–43.

[134] LaDu MJ, Lukens JR, Reardon CA, Getz GS. Association of human, rat, and rabbit apolipoprotein E with beta-amyloid. J Neurosci Res 1997;49: 9–18.
[135] Mahley RW, Huang Y. Apolipoprotein E: from atherosclerosis to Alzheimer's disease and beyond. Curr Opin Lipidol 1999;10:207–17.
[136] Poirier J, Davignon J, Bouthillier D, Kogan S, Bertrand P, Gauthier S. Apolipoprotein E polymorphism and Alzheimer's disease. Lancet 1993;342: 697–9.
[137] Strittmatter WJ, Weisgraber KH, Huang DY, Dong LM, Salvesen GS, Pericak-Vance M, et al. Binding of human apolipoprotein E to synthetic amyloid beta peptide: isoform-specific effects and implications for late-onset Alzheimer disease. Proc Natl Acad Sci USA 1993;90:8098–102.
[138] Refolo LM, Fillit HM. Apolipoprotein E4 as a target for developing new therapeutics for Alzheimer's disease. J Mol Neurosci 2004;23:151–5.
[139] Jofre-Monseny L, Minihane AM, Rimbach G. Impact of apoE genotype on oxidative stress, inflammation and disease risk. Mol Nutr Food Res 2008;52:131–45.
[140] Mahley RW. Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. Science 1988;240:622–30.
[141] Hatters DM, Peters-Libeu CA, Weisgraber KH. Apolipoprotein E structure: insights into function. Trends Biochem Sci 2006;31:445–54.
[142] Campos E, Nakajima K, Tanaka A, Havel RJ. Properties of an apolipoprotein E-enriched fraction of triglyceride-rich lipoproteins isolated from human blood plasma with a monoclonal antibody to apolipoprotein B-100. J Lipid Res 1992;33:369–80.
[143] Heeren J, Niemeier A, Merkel M, Beisiegel U. Endothelial-derived lipoprotein lipase is bound to postprandial triglyceride-rich lipoproteins and mediates their hepatic clearance in vivo. J Mol Med 2002;80:576–84.
[144] Krapp A, Ahle S, Kersting S, Hua Y, Kneser K, Nielsen M, et al. Hepatic lipase mediates the uptake of chylomicrons and beta-VLDL into cells via the LDL receptor-related protein (LRP). J Lipid Res 1996;37:926–36.
[145] Mamo JC, Bowler A, Elsegood CL, Redgrave TG. Defective plasma clearance of chylomicron-like lipid emulsions in Watanabe heritable hyperlipidemic rabbits. Biochim Biophys Acta 1991;1081:241–5.
[146] Saito H, Dhanasekaran P, Baldwin F, Weisgraber KH, Phillips MC, Lund-Katz S. Effects of polymorphism on the lipid interaction of human apolipoprotein E. J Biol Chem 2003;278:40723–9.
[147] Libeu CP, Lund-Katz S, Phillips MC, Wehrli S, Hernaiz MJ, Capila I, et al. New insights into the heparan sulfate proteoglycan-binding activity of apolipoprotein E. J Biol Chem 2001;276:39138–44.
[148] Marz W, Hoffmann MM, Scharnagl H, Fisher E, Chen M, Nauck M, et al. Apolipoprotein E2 (Arg136 → Cys) mutation in the receptor binding domain of apoE is not associated with dominant type III hyperlipoproteinemia. J Lipid Res 1998;39:658–69.
[149] Minnich A, Weisgraber KH, Newhouse Y, Dong LM, Fortin LJ, Tremblay M, et al. Identification and characterization of a novel apolipoprotein E variant, apolipoprotein E3' (Arg136 → His): association with mild dyslipidemia and double pre-beta very low density lipoproteins. J Lipid Res 1995;36:57–66.
[150] Horie Y, Fazio S, Westerlund JR, Weisgraber KH, Rall Jr SC. The functional characteristics of a human apolipoprotein E variant (cysteine at residue 142) may explain its association with dominant expression of type III hyperlipoproteinemia. J Biol Chem 1992;267:1962–8.
[151] Ji ZS, Fazio S, Mahley RW. Variable heparan sulfate proteoglycan binding of apolipoprotein E variants may modulate the expression of type III hyperlipoproteinemia. J Biol Chem 1994;269:13421–8.
[152] Mann WA, Meyer N, Weber W, Meyer S, Greten H, Beisiegel U. Apolipoprotein E isoforms and rare mutations: parallel reduction in binding to cells and to heparin reflects severity of associated type III hyperlipoproteinemia. J Lipid Res 1995;36:517–25.
[153] Shuvaev VV, Laffont I, Siest G. Kinetics of apolipoprotein E isoforms-binding to the major glycosaminoglycans of the extracellular matrix. FEBS Lett 1999;459:353–7.
[154] Elsegood CL, Pal S, Roach PD, Mamo JC. Binding and uptake of chylomicron remnants by primary and THP-1 human monocyte-derived macrophages: determination of binding proteins. Clin Sci (Lond) 2001;101:111–9.
[155] Kayden HJ, Maschio F, Traber MG. The secretion of apolipoprotein E by human monocyte-derived macrophages. Arch Biochem Biophys 1985;239:388–95.
[156] Lestavel S, Fruchart JC. Lipoprotein receptors. Cell Mol Biol (Noisy-le-grand) 1994;40:461–81.
[157] Hinagata J, Kakutani M, Fujii T, Naruko T, Inoue N, Fujita Y, et al. Oxidized LDL receptor LOX-1 is involved in neointimal hyperplasia after balloon arterial injury in a rat model. Cardiovasc Res 2006;69:263–71.
[158] Altenburg M, Johnson L, Wilder J, Maeda N. Apolipoprotein E4 in macrophages enhances atherogenesis in a low density lipoprotein receptor-dependent manner. J Biol Chem 2007;282:7817–24.
[159] Yu KC, Mamo JC. Chylomicron-remnant-induced foam cell formation and cytotoxicity: a possible mechanism of cell death in atherosclerosis. Clin Sci (Lond) 2000;98:183–92.
[160] Orr CF, Rowe DB, Halliday GM. An inflammatory review of Parkinson's disease. Prog Neurobiol 2002;68:325–40.
[161] Darley-Usmar V, Wiseman H, Halliwell B. Nitric oxide and oxygen radicals: a question of balance. FEBS Lett 1995;369:131–5.
[162] McGeer PL, McGeer EG. The inflammatory response system of brain: implications for therapy of Alzheimer and other neurodegenerative diseases. Brain Res Brain Res Rev 1995;21:195–218.
[163] Chen S, Frederickson RC, Brunden KR. Neuroglial-mediated immunoinflammatory responses in Alzheimer's disease: complement activation and therapeutic approaches. Neurobiol Aging 1996;17:781–7.
[164] Floyd RA. Neuroinflammatory processes are important in neurodegenerative diseases: an hypothesis to explain the increased formation of reactive oxygen and nitrogen species as major factors involved in neurodegenerative disease development. Free Radic Biol Med 1999;26:1346–55.
[165] Ruiz J, Kouiavskaia D, Migliorini M, Robinson S, Saenko EL, Gorlatova N, et al. The apoE isoform binding properties of the VLDL receptor reveal marked differences from LRP and the LDL receptor. J Lipid Res 2005;46:1721–31.
[166] Nelson DL, Cox MM. Lehninger principles of biochemistry. 4th ed. New York: W.H. Freeman and Company; 2005.
[167] van Horssen J, Wesseling P, van den Heuvel LP, de Waal RM, Verbeek MM. Heparan sulphate proteoglycans in Alzheimer's disease and amyloid-related disorders. Lancet Neurol 2003;2:482–92.
[168] Flood C, Gustafsson M, Richardson PE, Harvey SC, Segrest JP, Boren J. Identification of the proteoglycan binding site in apolipoprotein B_{48}. J Biol Chem 2002;277:32228–33.
[169] Bame KJ, Danda J, Hassall A, Tumova S. ***Abeta(1–40) prevents heparanase-catalyzed degradation of heparan sulfate glycosaminoglycans and proteoglycans in vitro. A role for heparan sulfate proteoglycan turnover in Alzheimer's disease. J Biol Chem 1997;272:17005–11.
[170] Skalen K, Gustafsson M, Rydberg EK, Hulten LM, Wiklund O, Innerarity TL, et al. Subendothelial retention of atherogenic lipoproteins in early atherosclerosis. Nature 2002;417:750–4.
[171] Gustafsson M, Boren J. Mechanism of lipoprotein retention by the extracellular matrix. Curr Opin Lipidol 2004;15:505–14.
[172] Bandtlow CE, Zimmermann DR. Proteoglycans in the developing brain: new conceptual insights for old proteins. Physiol Rev 2000;80:1267–90.
[173] Iozzo RV. Matrix proteoglycans: from molecular design to cellular function. Annu Rev Biochem 1998;67:609–52.
[174] Small DH, Mok SS, Williamson TG, Nurcombe V. Role of proteoglycans in neural development, regeneration, and the aging brain. J Neurochem 1996;67:889–99.
[175] Olofsson SO, Boren J. Apolipoprotein B: a clinically important apolipoprotein which assembles atherogenic lipoproteins and promotes the development of atherosclerosis. J Intern Med 2005;258:395–410.
[176] Kunjathoor VV, Chiu DS, O'Brien KD, LeBoeuf RC. Accumulation of biglycan and perlecan, but not versican, in lesions of murine models of atherosclerosis. Arterioscler Thromb Vasc Biol 2002;22:462–8.
[177] O'Brien KD, Olin KL, Alpers CE, Chiu W, Ferguson M, Hudkins K, et al. Comparison of apolipoprotein and proteoglycan deposits in human coronary atherosclerotic plaques: colocalization of biglycan with apolipoproteins. Circulation 1998;98:519–27.
[178] Olin KL, Potter-Perigo S, Barrett PH, Wight TN, Chait A. Biglycan, a vascular proteoglycan, binds differently to HDL2 and HDL3: role of apoE. Arterioscler Thromb Vasc Biol 2001;21:129–35.
[179] O'Brien KD, Lewis K, Fischer JW, Johnson P, Hwang JY, Knopp EA, et al. Smooth muscle cell biglycan overexpression results in increased lipoprotein retention on extracellular matrix: implications for the retention of lipoproteins in atherosclerosis. Atherosclerosis 2004;177:29–35.
[180] Tao Z, Smart FW, Figueroa JE, Glancy DL, Vijayagopal P. Elevated expression of proteoglycans in proliferating vascular smooth muscle cells. Atherosclerosis 1997;135:171–9.
[181] Manders EM, Stap J, Brakenhoff GJ, van Driel R, Aten JA. Dynamics of three-dimensional replication patterns during the S-phase, analysed by double labelling of DNA and confocal microscopy. J Cell Sci 1992;103(Pt 3):857–62.
[182] Castillo GM, Ngo C, Cummings J, Wight TN, Snow AD. Perlecan binds to the beta-amyloid proteins (A beta) of Alzheimer's disease, accelerates A beta fibril formation, and maintains A beta fibril stability. J Neurochem 1997;69:2452–65.
[183] Snow AD, Kinsella MG, Parks E, Sekiguchi RT, Miller JD, Kimata K, et al. Differential binding of vascular cell-derived proteoglycans (perlecan, biglycan, decorin, and versican) to the beta-amyloid protein of Alzheimer's disease. Arch Biochem Biophys 1995;320:84–95.
[184] Isherwood SG, Williams CM, Gould BJ. Apolipoprotein B-48 as a marker for chylomicrons and their remnants: studies in the postprandial state. Proc Nutr Soc 1997;56:497–505.
[185] van Greevenbroek MM, de Bruin TW. Chylomicron synthesis by intestinal cells in vitro and in vivo. Atherosclerosis 1998;141(Suppl. 1):S9–16.
[186] Caramelli P, Nitrini R, Maranhao R, Lourenco AC, Damasceno MC, Vinagre C, et al. Increased apolipoprotein B serum concentration in Alzheimer's disease. Acta Neurol Scand 1999;100:61–3.
[187] Pal S, Semorine K, Watts GF, Mamo J. Identification of lipoproteins of intestinal origin in human atherosclerotic plaque. Clin Chem Lab Med 2003;41:792–5.
[188] Proctor SD, Mamo JC. Intimal retention of cholesterol derived from apolipoprotein B_{100}- and apolipoprotein B_{48}-containing lipoproteins in carotid arteries of Watanabe heritable hyperlipidemic rabbits. Arterioscler Thromb Vasc Biol 2003;23:1595–600.

[189] Bereczki E, Gonda S, Csont T, Korpos E, Zvara A, Ferdinandy P, et al. Overexpression of biglycan in the heart of transgenic mice. An antibody microarray study. J Proteome Res 2007;6:854–61.
[190] Haag MD, Hofman A, Koudstaal PJ, Stricker BH, Breteler MM. Statins are associated with a reduced risk of Alzheimer disease regardless of lipophilicity. The Rotterdam study. J Neurol Neurosurg Psychiatry 2009;80:13–7.
[191] McGuinness B, Craig D, Bullock R, Passmore P. Statins for the prevention of dementia. Cochrane Database Syst Rev 2009;15: CD003160. Review. PMID: 19370582.
[192] Rosenberg PB, Mielke MM, Tschanz J, Cook L, Corcoran C, Hayden KM, et al. Effects of cardiovascular medications on rate of functional decline in Alzheimer disease. Am J Geriatr Psychiatry 2008;16:883–92.