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

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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.

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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

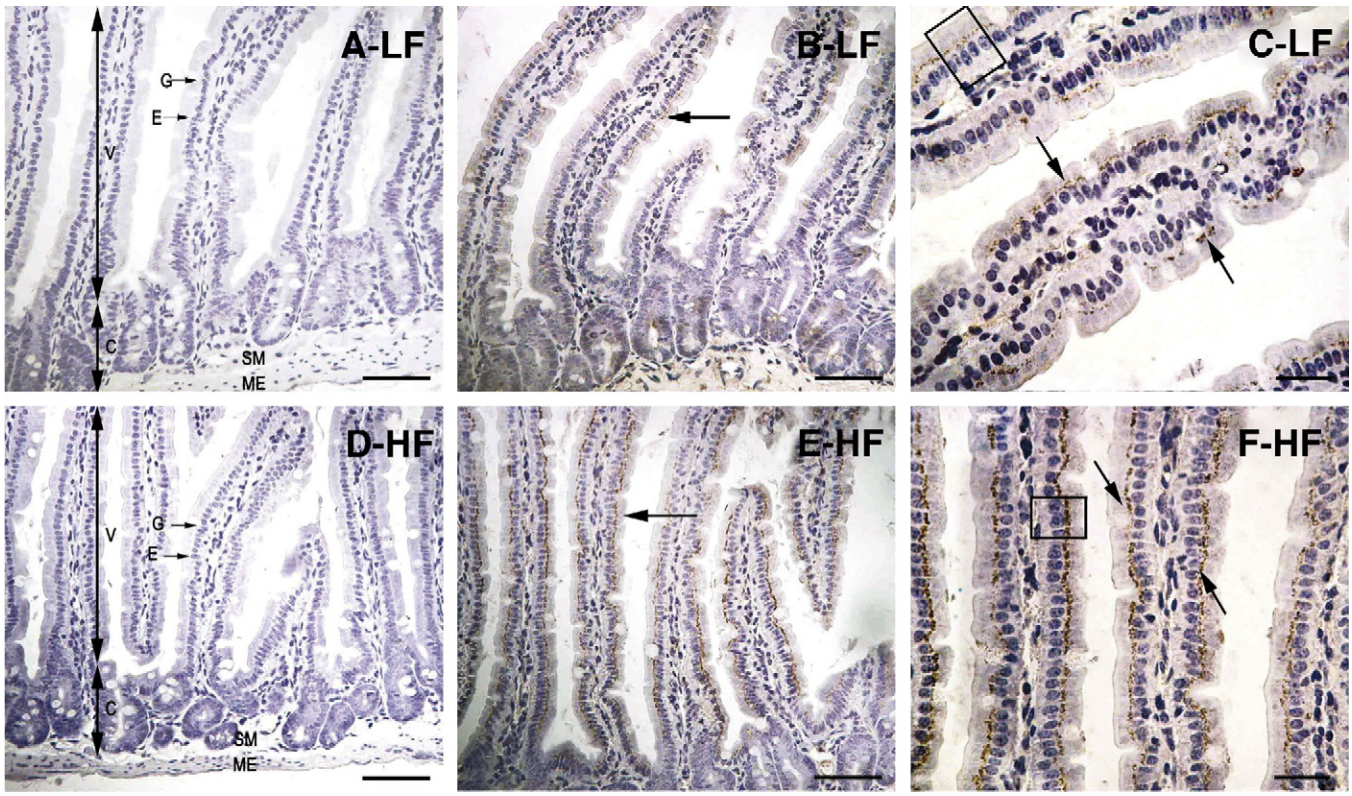

Fig. 1. Immunohistochemical detection of Aβ/APP in enterocytes of WT mice fed either a low-fat or a high-fat diet for 6 months. Intestinal sections at increasing magnifications show the pattern of Aβ/APP immunoreactivity (arrows in B, C, E and F) in the mucosal epithelium in mice fed a low-fat diet (LF) 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).

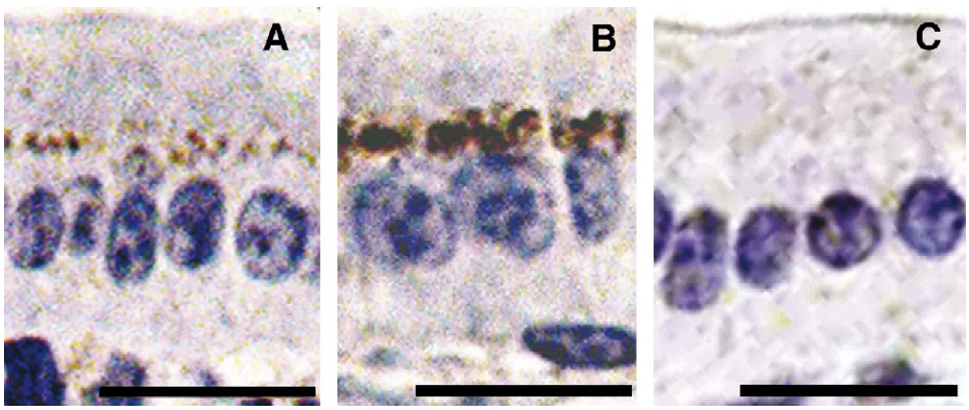

Fig. 2. Enterocytic expression of Aβ/APP in 6-month-old WT mice (C57BL/6J) fed a low-fat or a high-fat diet for 6 months or in mice fasted for 65 h. High magnifications of Aβ/APP immunostaining in enterocytes of the small intestine of mice fed a low-fat or a high-fat diet are shown in (A) and (B) (from 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.

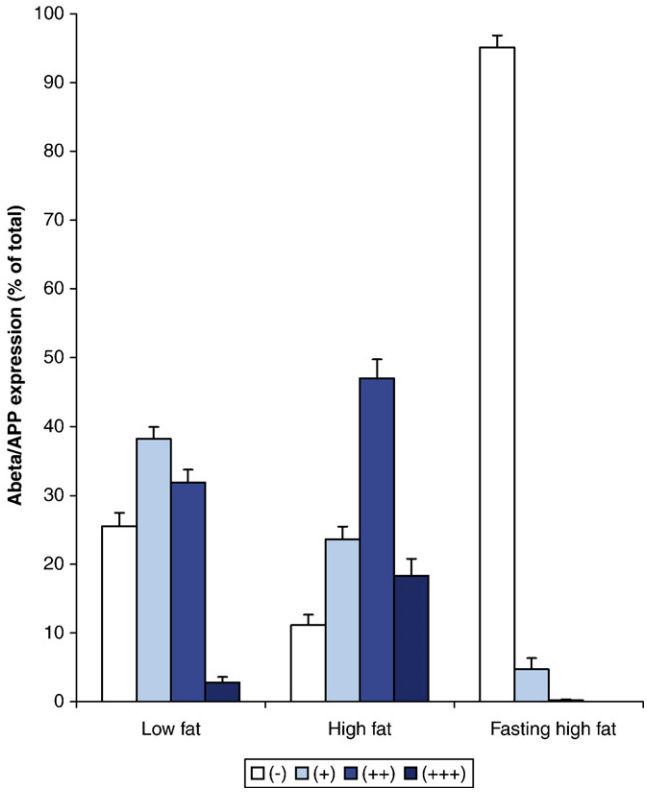

Fig. 3. Semiquantitative analyses of cellular Aβ/APP expressions in mice fed a high-fat or a low-fat diet and in mice fasted for 65 h (mice were initially given high-fat chow). Figure shows the proportion of cell numbers and the size of Aβ/APP expression between mice fed a high-fat diet and mice fed 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.